OCT 2 - 1933

VOLUME 23 NUMBER 9

SEPTEMBER, 1933

Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

CONTENTS

Pathologic Changes in the Anatomy of Leaves of the Sugar Beet, Beta vulgaris, affected by the Curly-Top Disease. KATHERINE ESAU 679

Culture of Phytophthora infestans ..... WILLARD CROSIER 713

Ascospore Discharge in Ophiobolus graminis, and it possible Relation to the Development of Whiteheads in Wheat. GEOFFREY SAMUEL and S. D. GARRETT 721

Weak Bordeaux Spray in the Control of Fire Blight. MONROE MCCOWN 729

Observations and Notes on Citrus Diseases in Minas Geraes, Brazil. ALBERT S. MÜLLER 734

Transmission of the Crinkle Disease of Strawberry ... E. K. VAUGHAN 738

Occurrence of Watermelon Mosaic ..... M. N. WALKER 741

Phytopathological Notes 745

Humus Extract Agar favorable for Oospore Production in Pythium ..... R. D. RANDS and ERNEST DOPP

A ring-spot-like Virus Disease of Red Clover ..... E. M. JOHNSON

Price { $6.00 per year, United State and dependencies,
$6.25 per year, Canada
$6.50 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

COR. LIME AND GREEN STREETS, LANCASTER, PA.

Entered at the post-office in Lancaster, Pa., as second-class matter.

DIRECTORY OF ADVERTISERS

LABORATORY EQUIPMENT AND SUPPLIES

BAUSCH & LOMB OPTICAL CO., 665 St. Paul St., Rochester, N. Y.	MICROSCOPES. MICROTOMES.
CLAY-ADAMS CO., 25 East 26th St., New York, N. Y.	SLIDES AND COVER GLASSES. MICROSCOPIC PROJECTION AND DRAWING APPARATUS.
DIFCO LABORATORIES, 920 Henry St., Detroit, Mich.	DEHYDRATED CULTURE MEDIA.
TRIARCH BOTANICAL PRODUCTS, Geo. H. Conant, Ripon, Wis.	PRESERVED PLANTS. MICROSCOPE SLIDES.
WARREN-KNIGHT CO., 136 N. 12th St., Philadelphia, Pa.	WRICO LETTERING GUIDES AND DRAFTING SUPPLIES.
ANSUL CHEMICAL CO., Marinette, Wis.	ANSUL FORMALDEHYDE DUST AND ANSUL COLLOIDAL SULPHUR.
THE GRASSELLI CHEMICAL CO., Cleveland, Ohio.	SPRAY MATERIALS AND DUSTS.
KOPPERS PRODUCTS CO., Koppers Building, Pittsburgh, Pa.	FLOTATION SULPHUR. PASTE, DRY WETTABLE, DUST.
ROHM & HAAS CO. INC., 222 W. Washington Square, Philadelphia, Pa.	RED COPPER OXIDE. FORM-O-FUME FORMALDEHYDE DUST.

SCIENTIFIC BOOKS AND PUBLISHERS

AMERICAN SOCIETY OF AGRONOMY J. D. Luckett, Editor, Geneva, N. Y.	THE JOURNAL OF THE AMERICAN SOCIETY OF AGRONOMY.
BIOLOGICAL ABSTRACTS, University of Pennsylvania, Philadelphia, Pa., U. S. A.	BIOLOGICAL ABSTRACTS.
PHYTOPATHOLOGICAL CLASSICS, H. H. Whetzel Mgr., Cornell University, Ithaca, N. Y.	NUMBERS 1 AND 2. PHYTOPATHOLOGICAL CLASSICS.
SCIENCE PRESS, Lime and Green Sts., Lancaster, Pa.	PRINTERS OF SCIENTIFIC JOURNALS, BOOKS AND MONOGRAPHS.
JOHN WILEY & SONS, INC., 440 Fourth Ave., New York, N. Y.	J. C. ARTHUR. PLANT RUSTS.
THE H. W. WILSON CO., 950-972 University Ave., New York, N. Y.	CLEMENTS AND SHEAR. THE GENERA OF FUNGI.

FERTILIZERS

N. V. POTASH EXPORT M. Y., INC., 19 West 44th St., New York, N. Y.	AGRICULTURAL AND SCIENTIFIC BUREAU. POTASH FERTILIZER.

HOTELS

WESTMINSTER HOTEL, Copley Square, Boston, Mass.	HEADQUARTERS HOTEL FOR BOSTON MEETING.

PHYTOPATHOLOGY

VOLUME 23 NUMBER 9

SEPTEMBER, 1933

PATHOLOGIC CHANGES IN THE ANATOMY OF LEAVES OF THE SUGAR BEET, BETA VULGARIS L. AFFECTED BY CURLY TOP[1]

KATHERINE ESAU[2]

(Accepted for publication, Oct. 1, 1932)

INTRODUCTION

Curly top of the sugar beet, *Beta vulgaris* L., induces profound anatomical changes in the affected plants, as do many other virus diseases.

The geographical distribution of curly top, its host range, and its general effects on the host plants are well known. Numerous studies have been made on the life history of the insect vector of this disease, the beet leaf hopper, *Eutettix tenellus* (Baker), and on the biological relation between that carrier and the curly-top virus. The properties of the latter have been in part determined.

The anatomy and physiology of plants affected by curly top have received, however, small attention; and there is scanty information on the processes involved in producing the histologic abnormalities.

This is a study of some important anatomical changes induced in leaves of the sugar beet by the disease known as curly top. Chief attention was given to changes in tissues in and near the degenerating phloem, after necrosis. Such a study promised to yield a general understanding of the processes by which the visible symptoms are produced. The statements of other workers concerning phloem necrosis have been checked, and several points on the normal anatomy have been cleared. A suggestion has been made that degeneration of the phloem is probably the primary effect of the virus on the plant and that certain other pathologic alterations have a causal relationship to the disturbance in the phloem. An effort has been made to

[1] Revision of the thesis submitted in December, 1931, to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] Grateful acknowledgments are made to Dr. W. W. Robbins, under whom this study was made, for his helpful guidance and criticism; to Dr. A. S. Crafts for his valuable suggestions and interest in the work; to Dr. Eubanks Carsner and to Dr. M. W. Gardner for their helpful criticism of the manuscript; to Mr. W. C. Matthews for making the photomicrographs.

correlate the knowledge gained from this study with the conception of some workers on the localization of certain viruses in plant tissues.

ANATOMY OF PLANTS AFFECTED BY VIRUS DISEASES

The morbid anatomy and physiology of virus-diseased plants in general have been somewhat neglected, as compared with other phases of virus studies. Few investigations have aimed at a morphological and physiological interpretation of the changes involved, but the pathological anatomy has often been studied incidentally in the search for a causal factor of virus diseases. In view of Quanjer's (45) recent review of these investigations, a very brief discussion of literature on the anatomy of virus-diseased plants will be given here. It shows the general similarity between the pathologic effects of curly top and of other virus diseases.

The three fundamental types of anatomic changes occurring in virus-diseased plants are (1) degeneration of certain cells or groups of cells, leading to a necrosis, (2) hyperplasia preceded by hypertrophy, and (3) hypoplasia. Necrosis is frequently confined to the phloem, as in certain potato virus diseases (30, 45, 50); but it may also occur in other tissues (35, 36, 44, 45). It may be not only internal but external, as in certain tomato (67, 68), and tobacco (34) diseases. Hypertrophy and hyperplasia, which have been described less frequently, may lead to the formation of overgrowths (29, 41) and may be closely associated with necroses (29). The green areas in leaves affected by mosaics are considered hyperplastic by certain workers (20, 21, 46), whereas the chlorotic areas have been found to be hypoplastic (9, 16, 17, 20, 21, 31, 46). The stunting and dwarfing of organs or of whole plants, effects very common to virus diseases, also are hypoplasias, in Küster's (38) terminology. The three principal types of abnormalities are usually combined, a fact which shows that the pathologic effect is not confined to one type of tissue but involves the whole plant.

EFFECT OF CURLY TOP ON THE SUGAR BEET

The following decsription of curly-top symptoms in the beet is given according to Severin (55). The first constant symptom on leaves is the clearing of tissues along the small veins, which Severin designated as "transparent venation." It generally occurs on the youngest leaves and may sometimes be confined to a portion of a leaf. It may appear on the youngest leaf of a very young plant within 2 days after inoculation and may sometimes be the only symptom visible on a leaf. Occasionally, but not always, blister-like elevations may give early indication of infection. As the disease develops, the lower surface of the leaf becomes roughened, and the veins are distorted. At first, small elevations may be observed

along the veins; these later assume the shape of nipple-like papillae and knot-like swellings resembling galls, termed by Severin "wart-like protuberances." At this stage the leaves are dark green, thick, crisp, and brittle. Frequently a clear, viscid liquid exudes from the petioles or the veins on the dorsal surface of the leaf. Inward rolling of diseased leaves usually accompanies other symptoms but occurs also in some other diseases, and is, therefore, not a sure indication of curly top. The roots of severely diseased plants are stunted and are stimulated to increased production of laterals.

Carsner and Stahl (15) state that "the disease is characterized by a necrosis of the phloem throughout the vascular system, and in the advanced stages of the disease this necrosis shows in the roots as dark concentric rings." They found that the clear, viscous exudate from the diseased petioles contains "a complex sugar, probably saccharose." In two instances traces of reducing sugars were detected. The dry weight of the diseased tops was found to exceed that of the tops of healthy plants, a fact attributed to carbohydrates, probably sugars, accumulating in the diseased leaves. The escape of the exudate was explained by "high pressure developed by the abnormal accumulation of the sugars." Leaves, mature at the time of infection, remained free from symptoms but contained the curly-top virus, which was also present in the roots.

Mumford (43) reported in diseased leaves an increase in sugars, in the concentration of total solids, and in nonelectrolytes.

Smith and Boncquet (59) described lesions in the phloem of affected plants; these were found in all parts that showed macroscopic irregularities, and to some extent in those parts where symptoms could not yet be distinguished. The abnormal condition in the phloem could first be recognized by the denser appearance of contents of sieve tubes and companion cells in certain areas; then followed a "necrosis, collapse, discoloration, and often, in the end, development of new, irregularly formed, wound-healing cells in these areas."

Rawlins[3] observed a degeneration of the pericycle cells and "several rows of adjacent cells" in root tips from curly-top-diseased sugar beets.

MATERIAL AND METHODS

Material for study consisted of sugar beets of the morphologically uniform strain 2705g24—very susceptible to curly top—developed by W. W. Tracy of the United States Department of Agriculture; and of the commercial strain, Rabbethge and Giesecke's "Old Type." Part of the plants were grown in pots in the greenhouse; the others, in a field plot.

[3] Rawlins, T. E. Cytology and other studies of curly-top disease of the sugar beet. Unpublished thesis. Library, University of California, Berkeley, Calif. 1926.

To obtain diseased material, virus-carrying males of the beet leaf hopper, *Eutettix tenellus,* were caged upon individual leaves, usually 5 leaf hoppers to a plant, and were removed, as a rule, 72 hours later. The beets in the field plot also were inoculated artificially by means of leaf hoppers reared in cages. In the field the insects were not caged upon individual plants but were set free. Those used for inoculation were fed beforehand on sugar-beet plants affected by curly top of a form that Carsner (14) designates as severe. The experimental plants received no other special treatment. Both greenhouse and field-plot beets were entirely free from sugar-beet mosaic.

The material was killed and fixed for 48 hours in Karpechenko's weak chromo-acetic-formalin solution made up as follows: solution A: 65 cc water, 10 cc glacial acetic acid, 1 gram chromic acid; solution B: 65 cc water, 10 cc commercial formalin (40 per cent stock solution). Equal quantities of solutions A and B were mixed immediately before placing the material in the killing fluid. For the first 12 hours the material was kept in cold storage at 45° F. Butyl alcohol was used for dehydration and clearing. The material was left 24 hours in each change of the ethyl-butyl-alcohol mixtures—longer than suggested by Zirkle (71). The sections were cut 10 microns in thickness and stained with Heidenhain's iron-alum haematoxylin and with a counter stain of light green in clove oil. Fresh material was sectioned by hand and observed in either tap water or glycerine.

PATHOLOGIC CHANGES IN THE PHLOEM AND PERICYCLE REGION

Structure of the phloem and pericycle region in healthy leaves.—Necrosis, a constant symptom of beets affected by curly top, involves the phloem and the region external to it—the bundle cap. This fact raises the question as to the composition of the bundle cap and its relation, if any, to the phloem.

According to de Vries (70), a vascular bundle in a beet leaf consists of the following tissues (Figs. 2, A and 10, A): on the ventral side of the leaf, a small bundle sheath consisting of long, thick-wall cells; then primary xylem, a layer of secondary xylem, cambium, phloem, and finally a "bast sickle" composed of cells whose walls are collenchymatously thickened. These "bast" cells have oblique radial walls and, frequently, thin transverse ones. The last layer of cortical cells, that adjoining the vascular bundles, is a starch sheath, which, in young leaves, contains abundant starch. For all the bundles, there is usually one common starch sheath, the cells of which are smaller than those of the adjoining parenchyma of the cortex. No intercellular spaces occur between the starch sheath and the cells of the bundle cap. The midrib and larger lateral veins form ridges

protruding from the surface of the leaf blade, particularly on the dorsal side. These projections have a layer of collenchyma on both the ventral and the dorsal surfaces of the leaf (Fig. 10, A). In the petiole the collenchyma is confined to the ribs.

Artschwager (1) designates the bundle cap at the phloem end, as well as that at the xylem end of a bundle, as a sclerenchyma cap, but does not describe it in detail.

Structures known in botanical literature as "bundle caps" consist of fibrous cells and are commonly associated with vascular bundles in leaves. According to Fitting (28, p. 88), bundle sheaths, a special type of which is represented by bundle caps, do not belong to the vascular tissue. They may be of pericyclic or cortical origin (26, p. 256). Sometimes, however, sheaths may be assigned to the bundle itself (3, p. 318). The occurrence of sclerenchymatous pericycle is characteristic of Chenopodiaceae, although the sclerenchyma is frequently only weakly developed (60, p. 662).

This investigation showed that the bundle cap in beet leaves rarely exhibits lignification. Lignified cells in the cap have been found most frequently in the large bundles at the base of the petiole, but occasionally also in the midrib and the large lateral veins. Lignification, if it occurs at all, usually is confined to the outer layer of the cap, although lignified cells are sometimes present in the second and third layers. Cap cells, showing lignin reaction with phloroglucin and HCl, have distinct tertiary thickenings and oblique transverse walls. They appear as sclerenchymatous fibers; and their position, adjacent to the starch sheath, indicates a pericyclic origin.

The major portion of the bundle cap consists of several layers of cells with comparatively thick cellulose walls. The thickenings, being pronounced in the corners, give the tissue the appearance of collenchyma, as de Vries (70) has indicated. This tissue merges imperceptibly with that of the functioning phloem (Figs. 2, A and 4, A); nor is there any line of demarcation between these cells and those of the pericycle, which may later become lignified. A sharp line of demarcation appears, however, between the pericycle cells and the starch sheath (Figs. 2, A; 2, B; 10, A), cells of the latter having much thinner walls and containing starch. In longitudinal sections, the starch-sheath cells are much shorter than those of the bundle cap.

The pericycle does not form a massive tissue in the leaves. Very young bundles contain, adjacent to the starch-sheath mother cells, a row of cells that may be interpreted as pericycle. Then follows the primary phloem, whose first mature sieve tube may be readily discerned in transverse sections because it is pentagonal or hexagonal in outline and appears optically empty in imbedded material. In the following stages of bundle develop-

ment, radial divisions occur in the pericycle, permitting lateral expansion. Then the cells enlarge, and their walls become thicker. The changes occurring in the primary phloem involve obliteration of sieve tubes and probably of their companion cells. The parenchyma cells of the primary phloem enlarge like those of the pericycle, their walls becoming thicker, particularly in the corners, where remnants of obliterated elements are discerned. In larger bundles a few tangential divisions occur in the pericycle, especially in the median bundle; but on the average not more than one row of cells may be considered as pericycle, because traces of obliterated elements of primary phloem are frequently discernible, even in older bundles, one row of cells removed from the starch sheath.

As obliteration in primary phloem progresses, the cambium forms a secondary phloem. A considerable amount of secondary growth occurs in the petioles, midribs, and larger lateral veins of beet leaves. Obliteration takes place also in the secondary phloem (Figs. 2, A; 4, A; 7).

Obliteration in phloem of healthy leaves.—Obliteration of phloem elements is a normal process in many plants having secondary growth. In angiosperms, the sieve tubes, companion cells, and cambiform cells are reported to be subject to obliteration (8, 64). In gymnosperms the sieve tubes and closely associated ray and parenchyma cells become functionless and collapse (64). Seeliger (53) finds both sieve tubes and companion cells obliterated in the sugar-beet root. The obvious changes and loss of contents have been assumed to be primary causes in the process of obliteration, pressure exerted by the surrounding living elements being only a secondary factor (8, 3).

In sugar-beet leaves, obliteration apparently involves both sieve tubes and companion cells. In sieve tubes, various stages of the process have been clearly observed, making possible an interpretation of the origin of the bundle cap.

Figure 1 shows obliteration of phloem elements in a petiole of a healthy, young sugar-beet leaf. Figure 1, A, represents a section through a region of the functioning secondary phloem. Within the phloem, the sieve tubes may be recognized by scarcity of contents and by the presence of structures known as sieve-tube plastids. The latter, although typical of sieve tubes of most plants, have not been found in any other type of cells. They have been described as starch grains by Briosi (10) and Lecomte (40) and as leucoplasts by Strasburger (64), who states that they produce a certain kind of starch that stains wine-red with iodine. In beets these bodies appear spherical, stain as protoplasmic structures with Heidenhain's iron-alum haematoxylin, and brown with iodine. They usually are aggregated near the sieve plates. The companion cells may be recognized by their

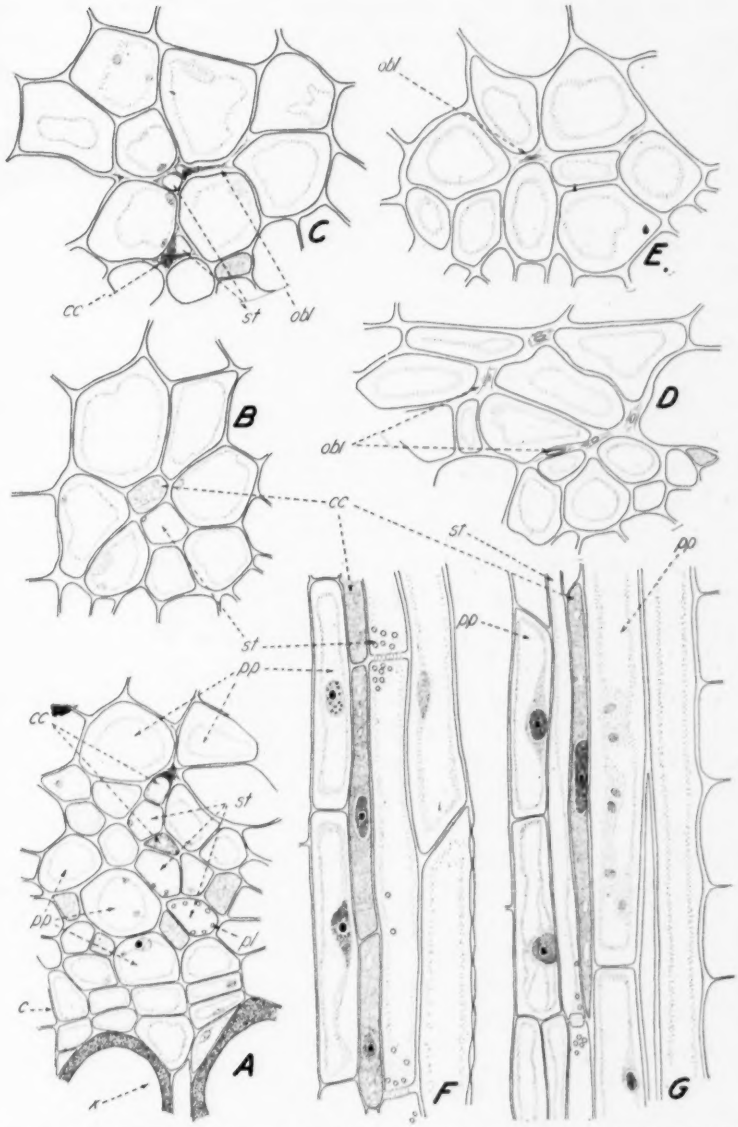

FIG. 1. Transverse (A–E) and longitudinal (F–G) sections of healthy phloem and bundle cap of petioles, showing obliteration of phloem elements. ×585. A and F. Functioning secondary phloem. B. Sieve tube and companion cell surrounded by enlarging phloem parenchyma cells. C. Early stage of obliteration. D and E. Advanced stages of obliteration. G. Early stage of obliteration; lumen of sieve tube decreasing, callus upon sieve plate increasing. Throughout figure: *st*, sieve tube; *cc*, companion cell; *pl*, sieve-tube plastid; *pp*, phloem parenchyma cell; *c*, cambium; *x*, xylem; *obl*, obliterated phloem elements.

position with respect to the sieve tubes and by their dense cytoplasm. In cross-section, the parenchyma cells of the functioning phloem are usually as large as the sieve tubes or larger; they contain a parietal layer of cytoplasm and some chloroplasts. Figure 1, B, shows a sieve tube and a companion cell surrounded by enlarging parenchyma cells. Figure 1, C, illustrates an early stage in the obliteration of sieve tubes and apparently of their companion cells. Some change has taken place, the contents of these elements being less abundant and the lumina of the cells having decreased in size. Figure 1, D, is drawn from the region of the primary phloem. Of the obliterated elements there remain only thickenings in walls between phloem parenchyma cells, with small, narrow slits indicating the position of the former lumina of the obliterated cells. In figure 1, E, no slits can be recognized in the region of obliteration. Figure 1, F, shows, in a longitudinal section, phloem parenchyma cells, a sieve tube with plastids, and companion cells from the region of the functioning phloem. Figure 1, G, represents an early stage in the obliteration of a sieve tube. The latter has become narrower, and the callus upon the sieve plate is more conspicuous than in figure 1, F.

The data on the structure of the phloem and bundle cap—the region that undergoes necrosis in curly-top-diseased sugar-beet leaves—may be summarized as follows. It is composed of the pericycle, the primary phloem, and the secondary phloem. In older leaves the primary phloem is represented only by phloem parenchyma, the sieve tubes and probably the companion cells having become obliterated. The primary phloem parenchyma and the pericycle cannot be distinguished readily, for they consist of similar cells and together, form a structure that appears as a bundle cap of collenchymatous elements located outside the secondary phloem. After the obliteration of sieve tubes in the secondary phloem the parenchyma of this phloem also becomes a part of the bundle cap. When Artschwager (1) states that "above the phloem is a large sclerenchyma "cap" and that "the phloem forms a narrow zone," he apparently includes in the "sclerenchyma cap" the parenchyma of the primary and of part of the secondary phloem.

Localization of necrosis in the phloem and pericycle.—Necrosis induced by curly top may sometimes be observed mainly in the secondary phloem (Fig. 2, B), may involve a portion of the cap (Fig. 3, A), or may transform the whole bundle cap into a large necrotic pocket (Fig. 2, C). In other words, in some bundles degeneration occurs chiefly in the secondary phloem (Fig. 2, B), while in others the primary phloem and pericycle regions are necrotic (Figs. 2, C, and 3, A). In young leaves, developed some time after infection of the plant, the bundle cap is entirely deformed

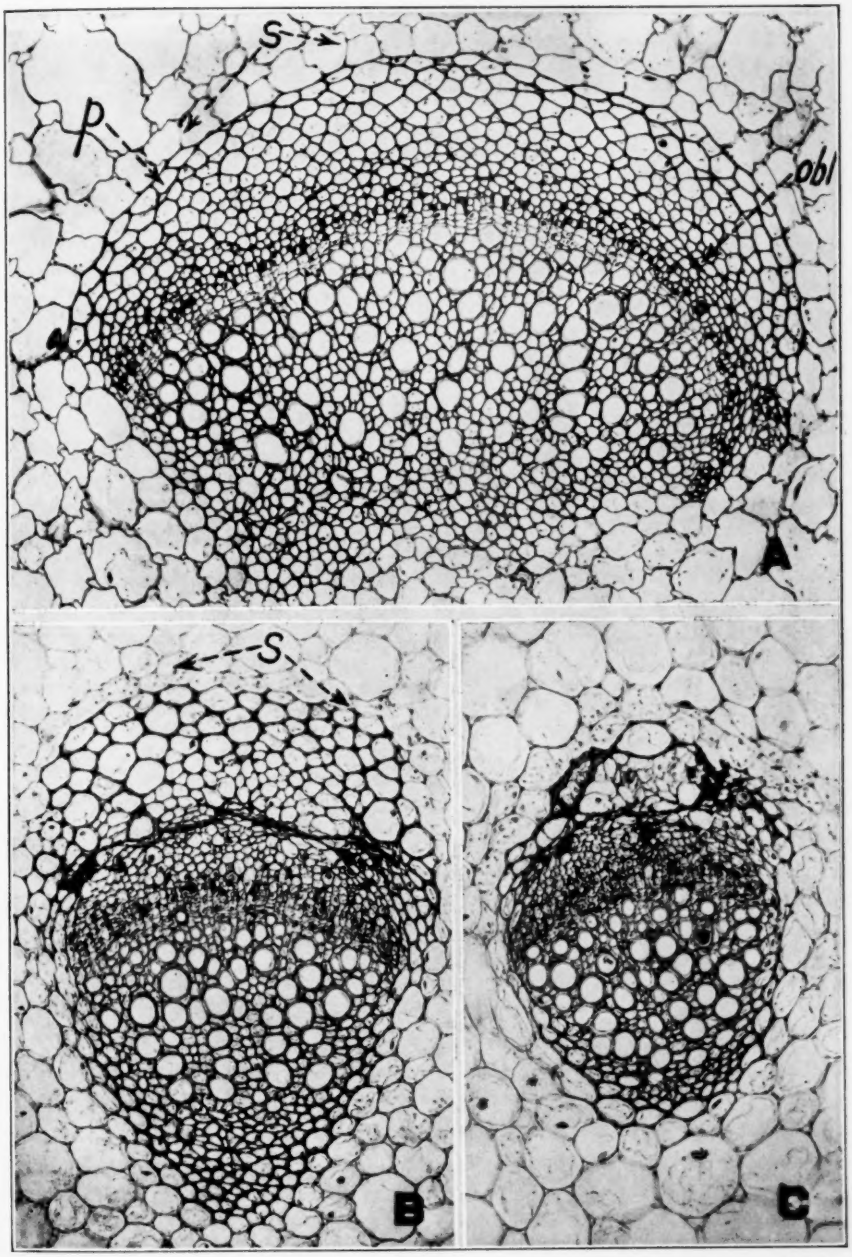

FIG. 2. Transverse sections of vascular bundles from petioles. ×125. A. Healthy. B. Diseased, showing necrosis in the secondary phloem. C. Diseased, showing complete destruction of bundle cap. Throughout figure. *p*, pericycle; *s*, starch sheath, *obl*, obliterated phloem elements.

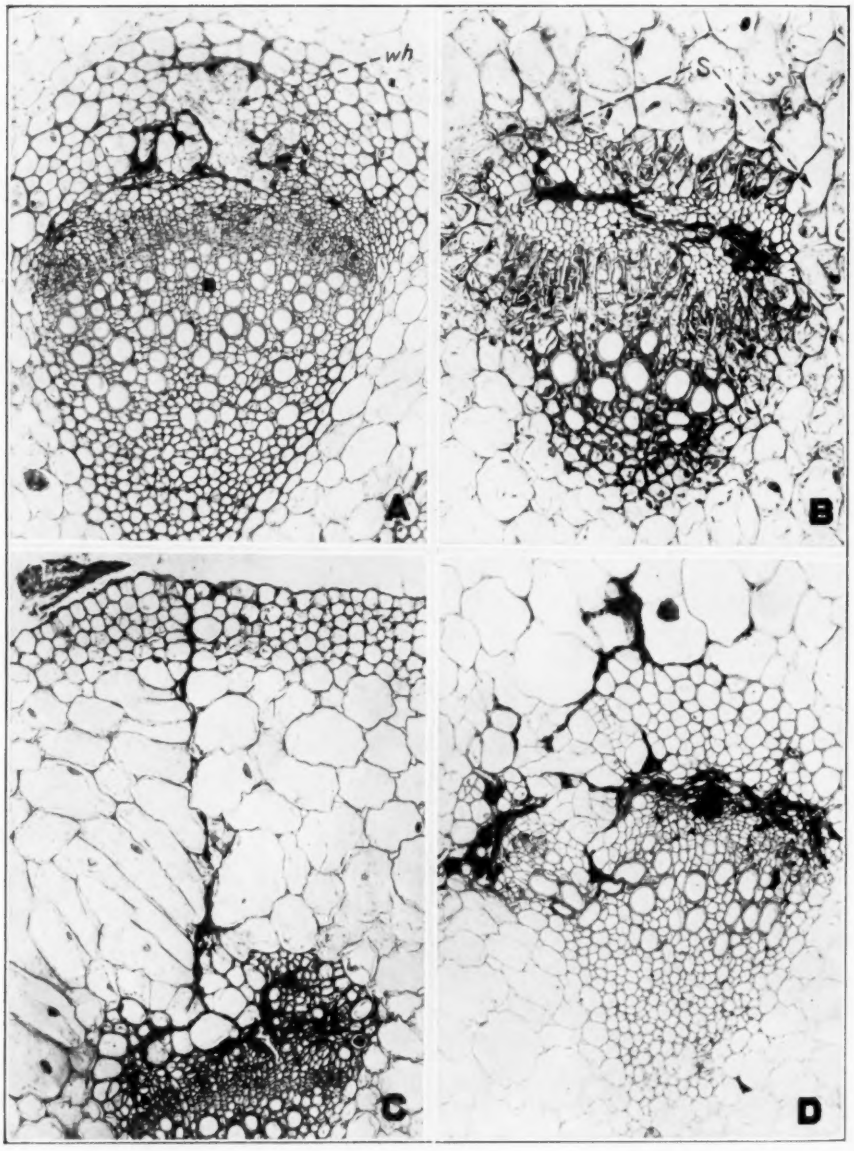

FIG. 3. Transverse sections of vascular bundles from petioles of a diseased plant. A. Bundle from mature leaf. The cap is partly destroyed. Cell proliferations, resembling wound-healing tissue (*wh*), have filled the cavity that resulted from collapse of certain cells. ×98. B. Bundle from young leaf. Cap deformed because of early hyperplasia in phloem and pericycle region. Line of demarcation between pericycle and starch sheath (*s*) very distinct. Necrotic area surrounded by hyperplastic tissue whose cell contents are scarce. ×200. C. Exudation of material from necrotic phloem to the exterior. Cortical cells along the path of the exudate show hypertrophy. ×98. D. Exudation of material from necrotic phloem into the xylem. Two vessels are partly crushed. ×98.

(Fig. 3, B), because the primary phloem and the pericycle have degenerated.

Necrosis has not been observed in any other tissue. In some cases material originating in the necrotic phloem may escape into the xylem, passing through the intercellular spaces and even filling some vessels (Fig. 3, D). It also may escape into the intercellular spaces in the cortex and exude to the exterior of the leaf (Fig. 3, C); but necrosis is apparent in neither the xylem nor the cortex. On the contrary, the parenchyma cells along the path of the exudate are stimulated to growth (Figs. 3, C, and D).

Necrosis of the phloem, as induced by curly top cannot be confused with normal obliteration. Necrotic areas in diseased plants are quite large (Fig. 4, B) as compared with the few cells involved in obliteration (Figs. 2, A, and 4, A). In the areas of obliteration, relatively inconspicuous thickenings of walls indicate the former position of the obliterated elements, whereas in the necrotic regions, collapsed walls form conspicuous layers, wavy and irregular in outline, and frequently showing traces of lumina of the dead cells. In necrotic areas accumulates a large amount of amorphous material, which stains deeply with coal-tar and haematoxylin dyes. No such accumulations occur in the region of obliteration. In fresh sections, the material in necrotic areas appears brown.

Hypertrophy and hyperplasia in the phloem.—Further studies must precede the interpretation of the stages in the degeneration of the phloem of beets affected by curly top. Preliminary studies indicate that, before the formation of lesions, hypertrophy and hyperplasia occur in the pericycle and phloem. Some cells divide in any plane; others hypertrophy without dividing, while their contents disintegrate.

In later stages certain cells collapse, thereby causing the appearance of lesions. In very young leaves collapse of cells does not result in a formation of cavities. The actively dividing cells probably exert a pressure upon the dying cells, hastening their collapse, and, afterward, form a small-cell, irregular tissue that surrounds the necrotic areas. These cells have dense cytoplasm at first; later, their contents become so scant that they stand out conspicuously among other tissue (Fig. 3, B).

In older leaves, necrosis leads to a formation of conspicuous cavities. The dark-staining material, which is present in lesions of recent origin, disappears later; and the cavities are closed by an irregular tissue resembling callus (Fig. 3, A). This tissue, having thin walls, may be readily differentiated from the hyperplastic tissue, just described for young leaves (Fig. 3, B). Evidently, both these types of tissue are a result of hyperplasia, but one begins to form before, the other after, the collapse of cells.

It would seem that the early hyperplasia is a response to some disturbance caused by the presence of the virus in the phloem, but the prolifera-

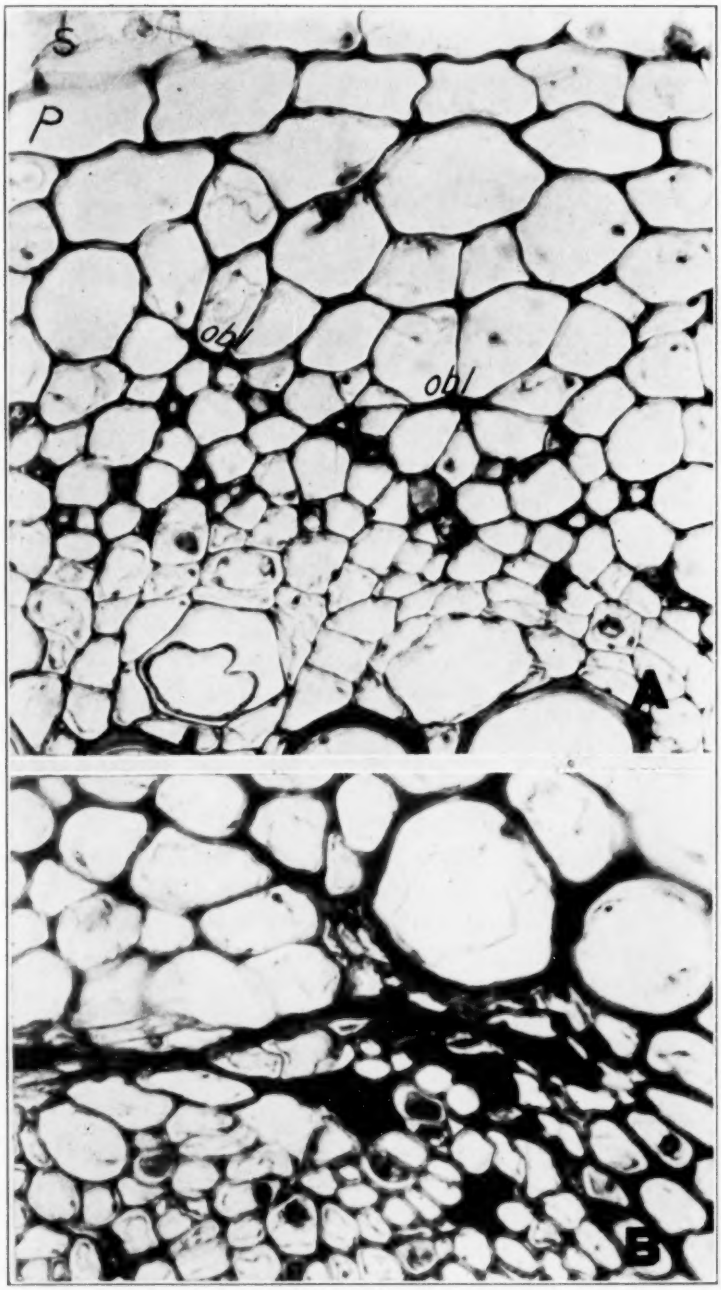

FIG. 4. Transverse sections of phloem of petiole. ×585. A. Healthy, showing obliterated phloem elements (*obl*), starch sheath (*s*), and pericycle (*p*). B. Diseased, showing collapsed walls and accumulation of deeply staining material in necrotic region.

tions connected with the lesions resemble wound-repair reactions. According to Küster (38, p. 76), cavities in a plant body, which result from death of cells, are similar to wounds, inasmuch as their presence induces the adjacent living cells to growth and divisions. The cavities in the phloem of curly-top diseased leaves resemble wounds, and the hyperplastic tissue, which closes these cavities, may conceivably have an origin similar to that of a wound-healing or callus tissue. Even if no conspicuous cavities are formed, hypertrophy and hyperplasia in tissues adjacent to necrotic areas may be comparable to repair or wound-healing reactions.

The phloem-parenchyma starch sheath and the cortical parenchyma have been observed to participate in the hypertrophic and hyperplastic processes in regions adjacent to the lesions in curly top. When necrosis does not involve the whole bundle cap, phloem parenchyma cells give rise to proliferations. This is the more frequent condition. When the whole cap is disrupted, the starch sheath cells may become meristematic and produce hyperplastic tissue. Other parenchyma cells may hypertrophy and divide near the lesions.

Figures 5 and 6 illustrate hypertrophy and hyperplasia in connection with the lesions in the phloem of a diseased leaf. Figures 5, A, and B, show callus-like tissue formed by phloem parenchyma. The intact starch sheath may be seen on the right, next to the lesion. In figure 5, A, the proliferated tissue is very irregular, while in figure 5, B, the cells appear to form a cambium. Figure 6 illustrates phloem parenchyma cells that have divided and filled a cavity. Figures 5, C, and D, show hypertrophy and hyperplasia of starch sheath cells. In figure 5, C, starch sheath cells still contain abundant plastids, but the cells have already elongated in a direction perpendicular to the surface of the cavity; the first cross walls have been laid down parallel to the surface of the cavity. In figure 5, D, the plastids have disappeared and the starch sheath cells have given rise to a small-cell, thin-wall tissue.

The filling of spaces by tissue produced through the division of neighboring cells is common in plants, even in their normal ontogeny. Wounding, whether external or internal, stimulates the adjacent living cells to growth and division (11, 38). The phenomena involved in this stimulation are not yet well understood. Some investigators would connect them with the physico-chemical changes caused by wounding or attribute them to the action of certain materials produced by necroses (38).

All living elements, if adjacent to wounds, may be stimulated to growth and division (38). Even cells having thick cellulose (collenchyma) or lignified (xylem-parenchyma) walls may participate in callus formation (19, 52), in which the cambium and the ground parenchyma are particularly efficient (38).

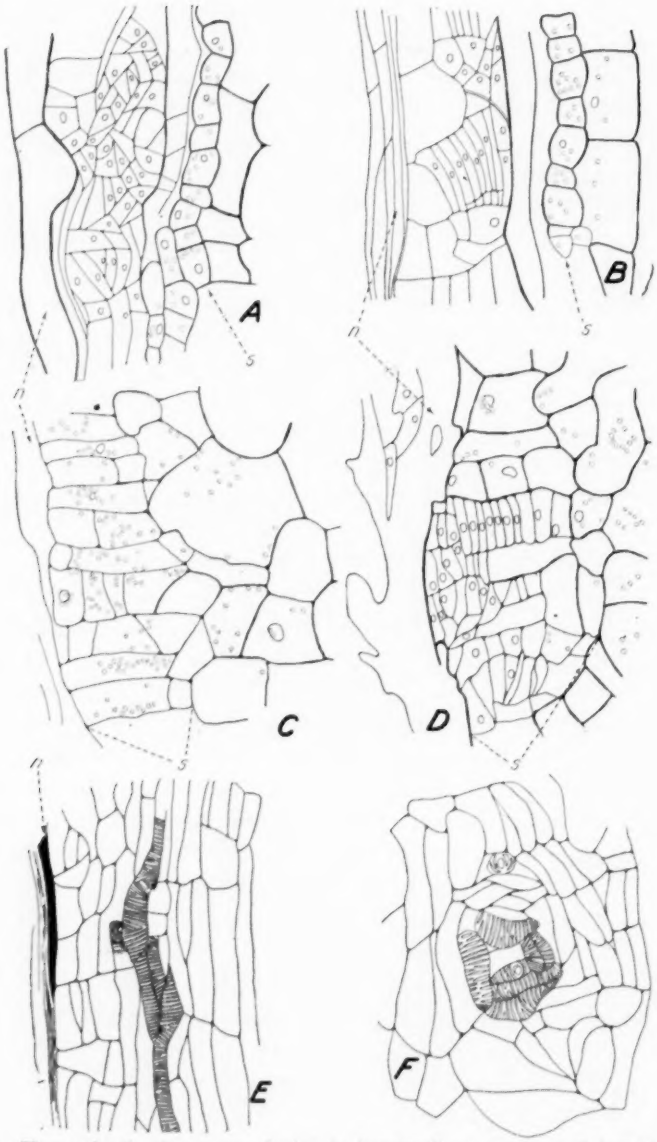

FIG. 5. Hyperplastic tissue near lesion in longitudinal sections of bundle cap from diseased leaf. A–E. ×147; F, ×239. A. Hyperplastic tissue formed by bundle-cap cells adjacent to necrotic area (*n*); starch sheath (*s*) intact; proliferated tissue irregular. B. As in A, but shows formation of cambium in proliferated tissue. C. Hypertrophy and early hyperplasia of starch-sheath cells (*s*) near necrotic region (*n*). D. Advanced hyperplasia of starch-sheath cells (*s*). E. Continuous row of xylem elements differentiated in hyperplastic tissue. F. Isolated ring-like group of xylem elements differentiated in the hyperplastic tissue.

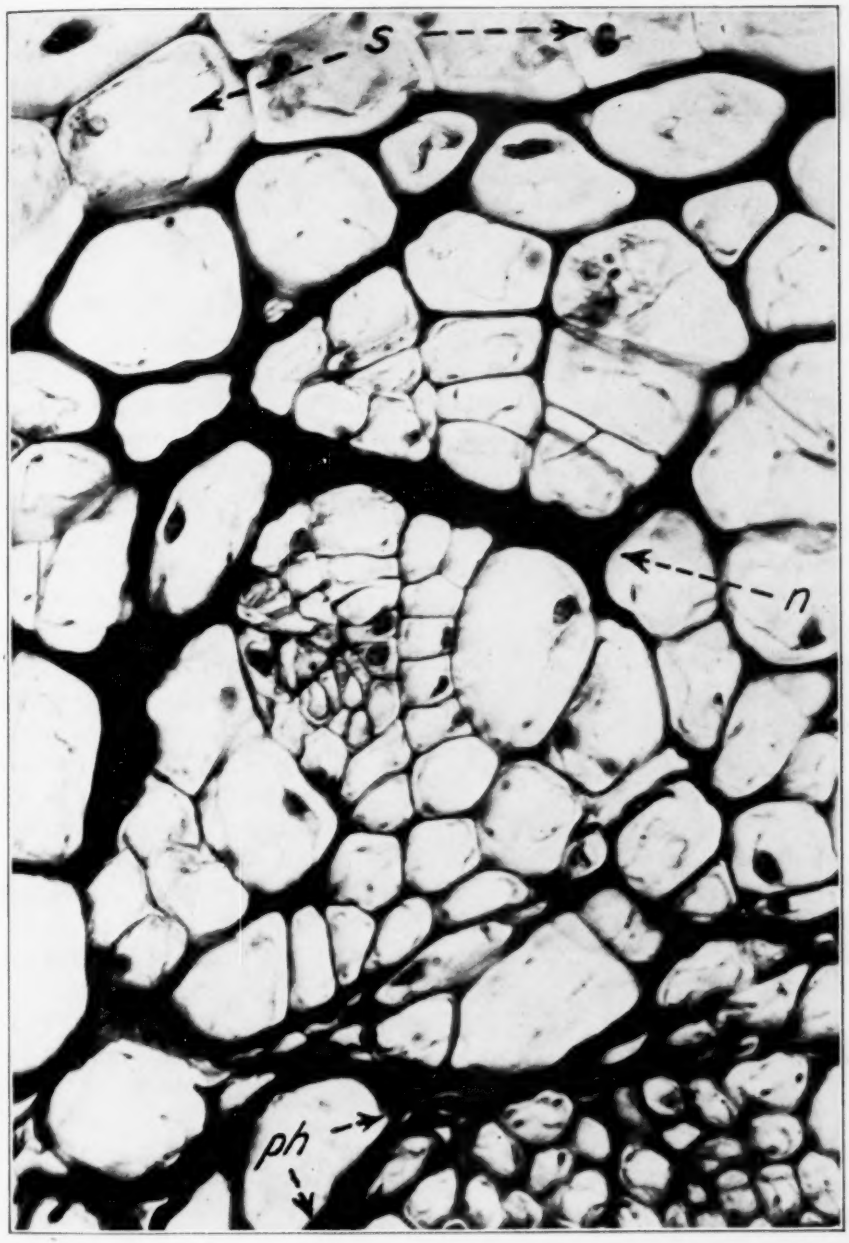

FIG. 6. Transverse section of diseased bundle cap, showing early stage of differentiation of hyperplastic tissue near lesion into vascular tissue. ×725. *ph*, phloem; *n*, necrotic area; *s*, starch sheath.

Phloem parenchyma in the sugar beet, being normally very active in producing meristematic tissue (53), may be readily stimulated to growth and division in the neighborhood of lesions in curly-top plants. The same is true of the pericycle, which undoubtedly participates in the filling of the cavities in diseased beet leaves. The starch sheath and cortex consist of parenchymatous cells, generally active in producing callus near wounds.

Frequently cork, instead of callus, is formed around wounds or lesions (11, 38, 42), but this has not been observed in curly-top diseased sugar beet leaves.

Secondary vascular tissue in the bundle cap of healthy leaves.—In sugar-beet leaves, severely affected by curly top, xylem elements frequently differentiate in the hyperplastic tissue in the degenerated bundle cap. This fact raises the question as to the possible relation of this xylem to the secondary cambium and vascular tissue normally produced in the pericycle and primary phloem region in roots and flowering stalks of healthy beets.

The sugar beet, like other members of the Chenopodiaceae, shows anomalous secondary growth caused by the function of supernumerary rings of secondary cambium. In the root, according to Seeliger (53), the first ring of cambium is formed in a normal way between the primary xylem and phloem. When this cambium begins to function, certain primary phloem parenchyma cells enlarge and divide, while the sieve tubes and their companion cells become obliterated. From primary-phloem cells is differentiated the first supernumerary ring of secondary cambium, which forms xylem elements and parenchyma centripetally and phloem elements and parenchyma centrifugally. Before the first phloem elements of this ring are differentiated, a cell is cut off centrifugally from the cambium initial, which becomes the initial for the second supernumerary ring of vascular tissue. This initial, in its turn, gives rise to the initial of the third supernumerary ring, and so forth. The rings of vascular tissue arising from the supernumerary cambiums appear in a cross-section of a beet root as concentric annular rings. In certain regions of the plant, the pericycle also participates in forming the initials of the secondary cambiums. In the root these initials arise mainly in the phloem parenchyma, as just described; in the upper part of the hypocotyl in the pericycle; and in the lower hypocotyl in both the phloem parenchyma and the pericycle. Although such is the usual situation, exceptions are very common: the pericycle and the primary phloem-parenchyma cells may substitute for one another very readily in all layers of the root and hypocotyl during the formation of meristematic tissue. Cambium may arise occasionally in the parenchyma cells of the secondary phloem. The pericycle and phloem-parenchyma cells have an unusual capacity for becoming meristematic;

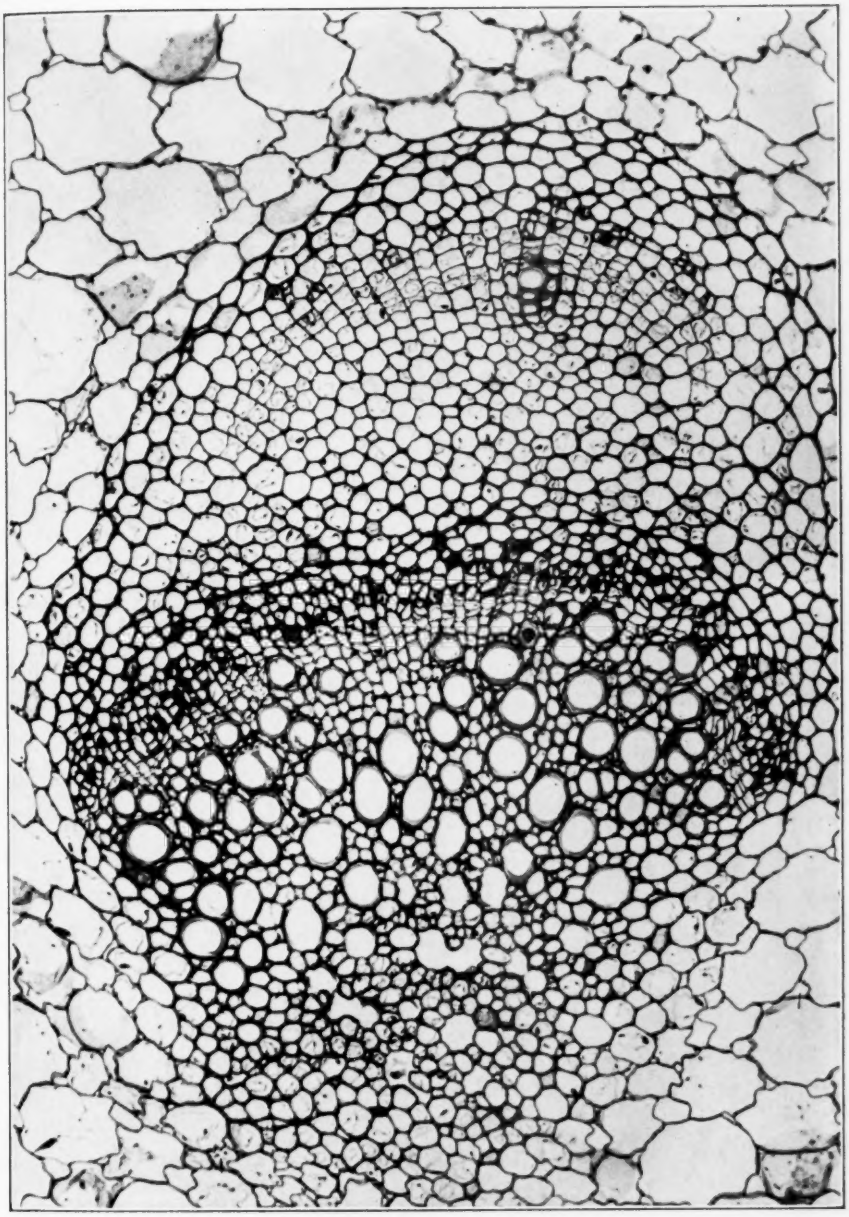

FIG. 7. Transverse section of a healthy bundle, showing formation of secondary cambium in bundle cap. First phloem and xylem elements have differentiated from this cambium. ×180.

being very slightly specialized, they may give rise to all types of cells that occur in a beet plant.

Artschwager (2) reported that in the floral axis the secondary cambium arises in the pericycle.

In this study, a large number of healthy young and old leaves, including those taken from flowering stalks, showed, upon examination, that secondary cambium forms to a limited extent in sugar beet leaves. Figure 2, A, represents a bundle without a secondary cambium, and figure 7 shows one, at the base of the petiole, in which a secondary cambium has arisen in the cap; from this cambium some phloem and several xylem elements have matured. Frequently, several of the larger bundles at the base of an old leaf show secondary vascular tissue in the cap.

In petioles the secondary cambium and the vascular tissue arising from it have been seen to form in the pericycle, but more frequently in the parenchyma of the primary phloem. They have been noted only in a short region at the base of the petiole. In cases studied, no such secondary vascular tissue was found at the insertion of the lamina or in the midrib or lateral veins.

Differentiation of xylem in the degenerated bundle cap of diseased leaves.—In the diseased leaves xylem differentiation in the cap occurs not only in the petiole, but in the midrib and lateral veins. This xylem is abnormal in its distribution and structure. It usually shows irregular distribution in both transverse and longitudinal sections (Figs. 8, A, and B). Cells of the callus-like tissue either differentiate directly into xylem elements or behave as cambium, forming radial rows of longitudinally elongated cells that mature into xylem elements. In the first case the xylem shows a very irregular distribution, frequently forming rings of xylem elements (Fig. 5, F) like those described by Küster (38) in the case of callus in Beta. These groups may have no connection with the rest of the abnormal xylem. In the second case, the abnormal xylem runs parallel to the normal and appears more regular (Fig. 5, E). Figure 6 shows an early stage in the development of vascular tissue in proliferated tissue near a lesion.

The normal xylem arising from a secondary cambium shows scalariform, scalariform-reticulate, and pitted elements and lignified fibers with unsymmetrical pits having narrow mouths. The abnormal xylem contains no fibers and consists of spiral, scalariform, and scalariform-reticulate elements.

Transverse and longitudinal sections of diseased leaves in no case revealed a connection between the xylem in the hyperplastic tissue and the normal xylem.

Leaves cleared in lactic acid (method by Simpson, 56) showed that the abnormal xylem may run parallel to the normal xylem through the petiole

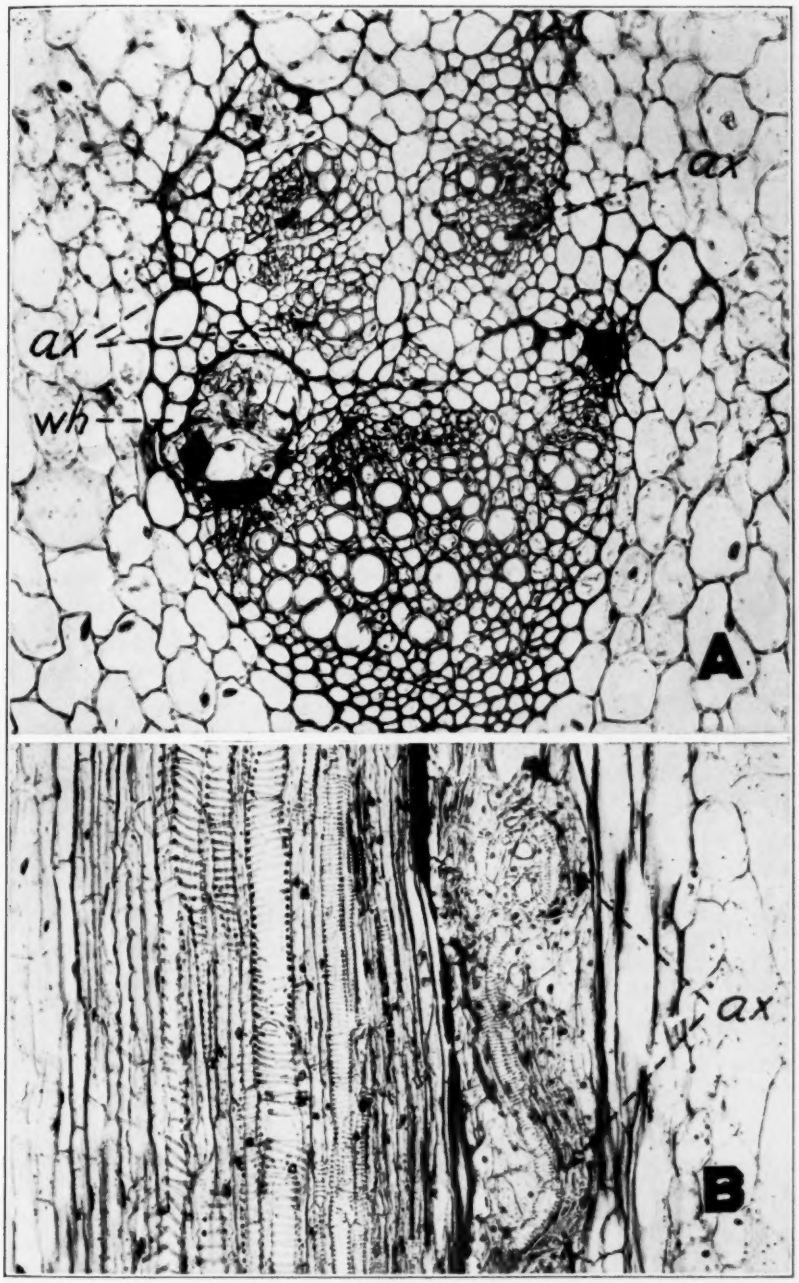

FIG. 8. Transverse (A) and longitudinal (B) sections of diseased bundle from the petiole, showing abnormal xylem (*ax*) and hyperplastic tissue near lesion (*wh*) in the bundle-cap region. ×174.

and midrib and into the lateral veins without forming any anastomoses with the normal xylem. This method also made it possible to distinguish islands of abnormal xylem that have no connection with the rest of the abnormal xylem.

The absence of connection between normal and abnormal xylem was also ascertained by introducing a stain into the xylem. This work was done under a dissecting binocular microscope, magnification $\times 50$, a concentrated solution of acid fuchsin in tap water being used. The studies were made on leaves severed from plants. At first a strand of abnormal xylem was freed with a dissecting scalpel from surrounding tissues in a small area $\frac{1}{2}$ in. above the base of the petiole. A drop of the stain was then placed upon the freed portion of the strand, which was being supported upon a scalpel. With a second scalpel the strand was then cut under the fuchsin solution. After 5 minutes or thereabouts, the cortical tissue was removed above the vascular strand all along its course, and the distribution of the stain was determined. It proved to remain confined to the abnormal xylem, which appeared as a red line upon the white background of the normal xylem underneath. The abnormal xylem itself was continuous, and the stain spread from the base of the petiole into the abnormal xylem of the lateral veins. In several cases tracheal tubes leading along the midvein would suddenly terminate in a ring-like group of elements; from this point on, only one tube would continue into the lateral vein. Introduction of the stain into the normal xylem only was also tried, the strand of abnormal xylem being in this case removed for a distance of approximately an inch at the base of the petiole. The latter was then immersed in acid fuchsin solution, and a small section cut off at the base. The stain entered the normal xylem but was not found in the clipped strand of abnormal xylem. The latter appeared as a white line upon the red background of stained normal xylem. The stain, when introduced into an individual normal xylem strand, was distributed through all the other normal strands but did not enter the abnormal xylem.

Whether or not phloem differentiates along with abnormal xylem has not been determined. The adjacent tissue often resembles phloem in cross-section, but no sieve tubes were definitely recognized in longitudinal sections.

The formation of xylem in the hyperplastic tissue of curly-top leaves is not an extraordinary phenomenon. Xylem and phloem commonly differentiate in callus (38), and, moreover, the tissues of Beta are readily stimulated by wounding to produce cambium and vascular tissue (69).

Exudate from necrotic phloem.—The previously mentioned exudate from diseased leaves was tested according to Flückiger as quoted by Tun-

mann (66, p. 184). Only traces of reducing sugars were observed. The Cu_2O precipitate was, however, abundant after inversion with HCl, a fact indicating that sucrose is the predominant carbohydrate constituent of the exudate. Similar results were observed by Carsner and Stahl (15), employing Fehling's test.

The microchemical tests on the exudate thus far made are not a sufficient basis for any conclusions regarding the proportions of carbohydrate components of the exudate. The fact, however, that the latter contains sugar apparently indicates that it is derived from the phloem, although, according to Ruhland (47), the sugar is translocated in beet leaves mainly in the form of invert sugar. The anatomical studies also show that the exudate may be traced back to the phloem region, from which it escapes through the intercellular spaces (Figs. 3, C, and D). The exudate is probably the material translocated in the phloem. According to Crafts (18) the material in healthy phloem is under high pressure, but remains confined to this tissue because of the presence of limiting layers (cambium on one side and the starch sheath on the other). In view of this theory, the suggestion might be made that necrosis of the phloem so affects the limiting layers that they cannot resist the pressure existing in the phloem, and the translocated material escapes into the xylem or to the exterior. On the other hand, there is a possibility that the pressure existing in the phloem is increased in diseased leaves (15) and that the material is forced into the intercellular spaces of surrounding tissues.

PATHOLOGIC CHANGES IN TISSUES OTHER THAN THE PHLOEM

Clearing of the veins.—At this point, a few words are necessary concerning the appropriateness of the expressions *clearing of the veins* and *transparent venation*. The term *vein* in botanical literature is used rather loosely. It is commonly applied both to the vascular tissue and to the tissues in which the vascular strands are imbedded and which, in the case of larger strands, form protrusions and ridges. In a more exact terminology, according to de Bary (3, p. 299), the term *vein, nerve,* or *rib* is applied only to the protrusion, that is, to the external formation of parts; while the *vascular bundle* or *strand* refers to the inner structure. The former loose usage of the term *vein,* being very convenient, has become established. There should be, therefore, no objection to the continued mention of clearing of veins, the more so as this expression describes a phenomenon that may be recognized externally. A substitution of *translucent* for *transparent* is to be recommended, because the veins in curly-top leaves do not permit objects to be seen through them (transparency), but they admit the passage of light (translucency) to a greater extent than the veins in healthy leaves.

Clearing of the veins has been described not only in curly-top sugar beets, but also in the case of plants affected by certain other virus diseases. In curly top, however, it appears not to be identical with clearing of the veins in some other diseases. In the case of crinkle-"A" mosaic disease of potato, the veins of the young leaves become bright yellow and necrotic (48, 49); in asters affected by yellows, only a slight yellowing appears along the veins (36). In curly-top beets, on the other hand, the veins become neither yellow nor necrotic, but appear translucent in transmitted light. This condition is not comparable to that termed "water soaked."

No investigator has given definite morphological and physiological description of clearing of veins. Kunkel (37) suggests that this symptom may result from a stimulation of cell growth in the phloem.

In curly-top leaves showing translucent veins, the small veinlets may be recognized in transmitted light. In a healthy leaf only the larger veins are somewhat translucent, because their protrusion consists of large cells containing few chloroplasts (Figs. 10, A, and 9, C), but the small veins are surrounded by mesophyll cells with a large number of chloroplasts.

Clearing of the veins is an early symptom and usually appears in all newly-developed leaves as long as the plant lives. Very soon, however, the translucent veins become swollen. In severely affected plants the translucent-venation stage is short, the veins in very young leaves becoming thickened and distorted. In less severely affected plants the swelling may be very slight or undiscernible with the naked eye. Blisters (55) resemble translucent venation except that the clearing and swelling are confined to isolated areas along the veins.

Changes in leaves that may be recognized at the stage of cleared veins involve both the phloem and the adjacent mesophyll. As previously mentioned, in the early stages of disease hypertrophy and hyperplasia occur in the phloem. These may be recognized in leaves showing translucent veins. The stimulation of cells to growth and division extends beyond the phloem, however, involving mesophyll cells adjacent to the bundle on the phloem side. These cells divide radially and become so tightly packed as to leave no intercellular spaces. The chloroplasts are few and pale, some showing irregular shape, which indicates a condition of degeneration. This stage is represented in figure 9, A. For comparison, a cross-section of a healthy leaf is represented in figure 9, B. The mesophyll here shows the same number and size of chloroplasts below and above the small bundle. Intercellular spaces are formed very early in the mesophyll of healthy leaves on both sides of the bundle, whereas, in the diseased leaf, such spaces are absent on the phloem side. This change occurs not only in cells adjacent to the phloem but extends to the epidermis on the dorsal side of the leaf

and laterally beyond the phloem region. In subsequent stages the cells near the phloem elongate radially and become distinctly larger than those farther removed.

Leaves showing clearing of veins were taken from 2 kinds of plants: those recently inoculated with the virus and just having developed trans-

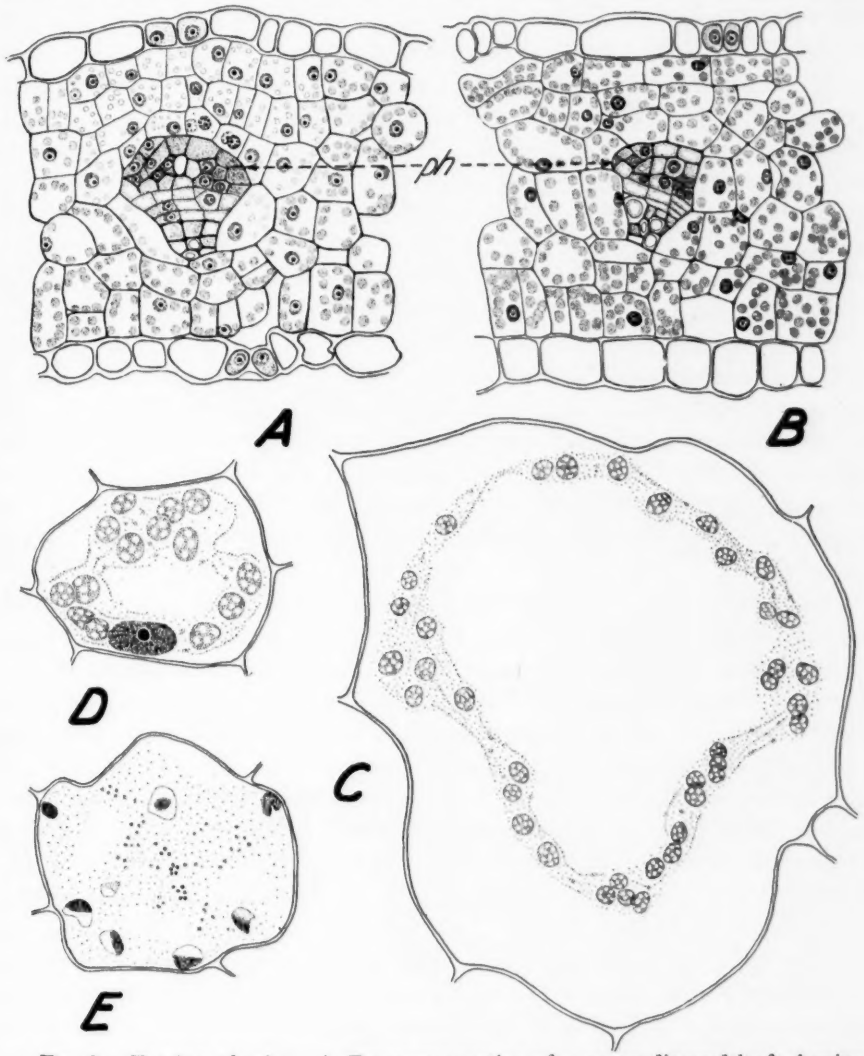

FIG. 9. Clearing of veins. A. Transverse section of a young diseased leaf, showing hyperplasia of cells and degeneration of chloroplasts in mesophyll adjacent to phloem (*ph*). ×450. B. Transverse section of a young healthy leaf. ×450. C. Parenchyma cell from a large vein of a young healthy leaf. ×1060. D. Healthy mesophyll cell from region adjacent to phloem of small bundle. ×1060. E. Diseased mesophyll cell from region adjacent to phloem of small bundle, showing degenerated chloroplasts. ×1060.

lucent veins or blisters; and those that had been infected for a long time but in which the youngest leaves showed pronounced translucent venation. The degeneration of chloroplasts was observed in both kinds of leaves.

In fresh sections appear degenerating pale green chloroplasts of irregular shape. Considerable hyaline granular material is present in cells where the chloroplasts have degenerated to such a stage that few or none are present in the cells. In fixed material the degenerating chloroplasts stain more weakly than normal ones, so that the regions about the phloem in stained sections appear pale. In such material the chloroplasts are of irregular shape and in many cases they appear as small bodies surrounded by a halo. The granular material also was recognized in stained sections. Figures 9, D, and 9, E, compare cells containing healthy and degenerated chloroplasts in stained sections. These cells were drawn from similar regions in the mesophyll near the phloem of a small vein in a healthy and a diseased leaf, respectively. Figure 9, C, shows a cell taken from a protrusion of a large vein. The cell is larger than the mesophyll cell in figure 9, D, but contains smaller chloroplasts, fewer in number per unit volume.

A marked feature of many of those cells showing degeneration of chloroplasts was an extremely rapid movement of small granules—too rapid to be accounted for by the protoplasmic streaming and apparently indicating some change in the structure of the cytoplasm. Dickson (20) observed a similar phenomenon in mesophyll cells of various plants affected by certain mosaics.

The degenerating cells were further characterized by the presence of numerous small vacuoles, as large as healthy chloroplasts or larger. Such vacuoles may occasionally be observed in healthy cells also, but much less frequently than in diseased. The vacuoles were not observed in the material embedded in paraffin, but appeared in sections treated with the killing solution (Karpechenko's weak) for a few minutes and then mounted in glycerine. In such material, the vacuoles could be made to show more distinctly by staining the sections with anilin blue. Numerous small granules, apparently, had accumulated at the surface of the vacuoles and, by absorbing blue stain, gave a sharp outline to the vacuoles. In fresh sections these granules appeared green.

The various phenomena described above have been observed also in the case of many other diseases. Translucent tissue with reduced intercellular space has been described in virus-diseased plants by several workers, among others by Dickson (20), Doolittle (21), Gardner (29) and Rand (46). The hypertrophy of green cells is usually accompanied by the disappearance of chloroplasts (38). Such cells may become totally depleted of chlorophyll, as in the case of intumescences (61).

Many virus diseases affect the chloroplasts and thus induce chloroses of various types. Some investigators report that the chloroplasts disintegrate (22, 27, 32, 46, 62); others attribute their reduction in size and number in certain mosaic diseases to inhibition of their development (9, 16, 17, 31, 35). In severe cases of certain diseases, the chloroplasts have been reported to disintegrate, while in milder cases of the same disease their development was only inhibited (20, 33). Apparently, both hypoplasia and disintegration of chloroplasts may take place in virus diseased plants, depending upon the stage of development at which an organ is infected as well as upon the severity of the disease.

Both phenomena probably occur in curly-top beets. The disintegration of chloroplasts was very distinct in leaves where they were well developed before hypertrophy began. In younger leaves containing very small chloroplasts, disintegration is less marked. In severely affected plants, hypertrophy along the veins may perhaps start so early as to inhibit the development of the chloroplasts.

Investigators report destruction of chloroplasts by living organisms (27, 62); dissolution (22); swelling (5, 6); and fragmentation (5, 6, 20, 32, 33, 46), which was the only phenomenon observed during these studies.

Vacuolation of cells, as observed in the case of virus, fungal, and bacterial diseases, has been interpreted as a sign of superactivity in the affected cells (23, 24, 25). This condition may also be induced by nonliving agents, both physical and chemical (39).

The findings of this investigation, considered in conjunction with the facts just cited, justify the conclusion that certain cells in translucent veins are in a stage of degeneration. Hypertrophy and hyperplasia, degenerative changes that, in the phloem, are somehow related to necrosis, in the mesophyll are accompanied by only partial destruction of cell contents. Clearing of the veins is a manifestation of the early stages of degeneration occurring in and along the vascular bundles. Although hypertrophy and hyperplasia may be recognized in the phloem at the stage of cleared veins, the translucency of veins seems to be explained mainly by enlargement, division, and close packing of mesophyll cells adjacent to the phloem, and by the reduction of their chlorophyll content.

Overgrowths on veins.—Veins of diseased leaves become thickened and distorted. Upon them appear numerous overgrowths of various sizes, some representing only slight irregularities on the surface, others developing into long papillae. Translucent veins, swollen veins, and wart-like protuberances are successive manifestations of one continuous process—the hypertrophy and hyperplasia of the parenchyma tissue about the phloem. At the translucent venation stage, as was mentioned above, division of cells,

followed by their radial elongation, occurs in the mesophyll adjacent to the phloem. Divisions in planes other than radial also occur during the development of a protuberance, but the radial elongation of cells is a more important cause of the extreme length of the protuberances. The long cells have but few chloroplasts and are surrounded by apparently normal cells containing a larger number of chloroplasts. The anatomical structure of healthy and of proliferated lateral veins is shown in figures 10, A, and 10, B.

Where hypertrophy and hyperplasia are not so extensive, the veins are merely thickened. In dwarfed, severely affected leaves, mesophyll other than that adjacent to bundles may be in a hypertrophied condition, resulting in abnormal thickness.

The overgrowths are always found in connection with degenerated phloem in the bundle. Either necrotic tissue or healed-over lesions may be associated with a protuberance. Townsend (65) does not make this clear in his illustration. The bundle associated with the overgrowth, as shown by him, does not differ from the healthy bundle. Abnormal xylem may be present in the degenerated phloem region (Fig. 10, B), a part of the abnormal xylem strand running parallel to the normal xylem strand in the lateral vein. No xylem occurs in the pericycle and phloem region of a healthy lateral vein.

A certain degree of hypoplasia is involved also in the formation of protuberances, no collenchyma developing below the epidermis of a protuberance, as in a healthy vein (Figs. 10, A, and 10, B). The xylem is also in a hypoplastic condition.

Consumption of cell contents and an early death do not occur in proliferations on sugar-beet leaves as they do in hyperhydric tissues. They have a certain similarity to tumors or galls induced by animal parasites, being more or less permanent structures, formed not only through hypertrophic but also through hyperplastic processes. The galls, however, show a more complex structure and frequently a more definite organization.

Proliferations, morphologically similar to the protuberances in curly-top sugar beets, may be induced in plants by mechanical and chemical means, as well as by other diseases and parasites. Schilling (51) induced overgrowths on certain plants by covering small areas on stems with paraffin. Smith (57, p. 477) obtained hyperplasias by treating plants susceptible to crown gall with chemicals said to be produced by the crown-gall organisms. According to this investigator, overgrowths may also be induced by slight freezing, mechanical irritation, over-watering in a confined atmosphere, semi-asphyxiation, etc. Although the agents that stimulate the production of hyperplasias are widely different, they produce similar

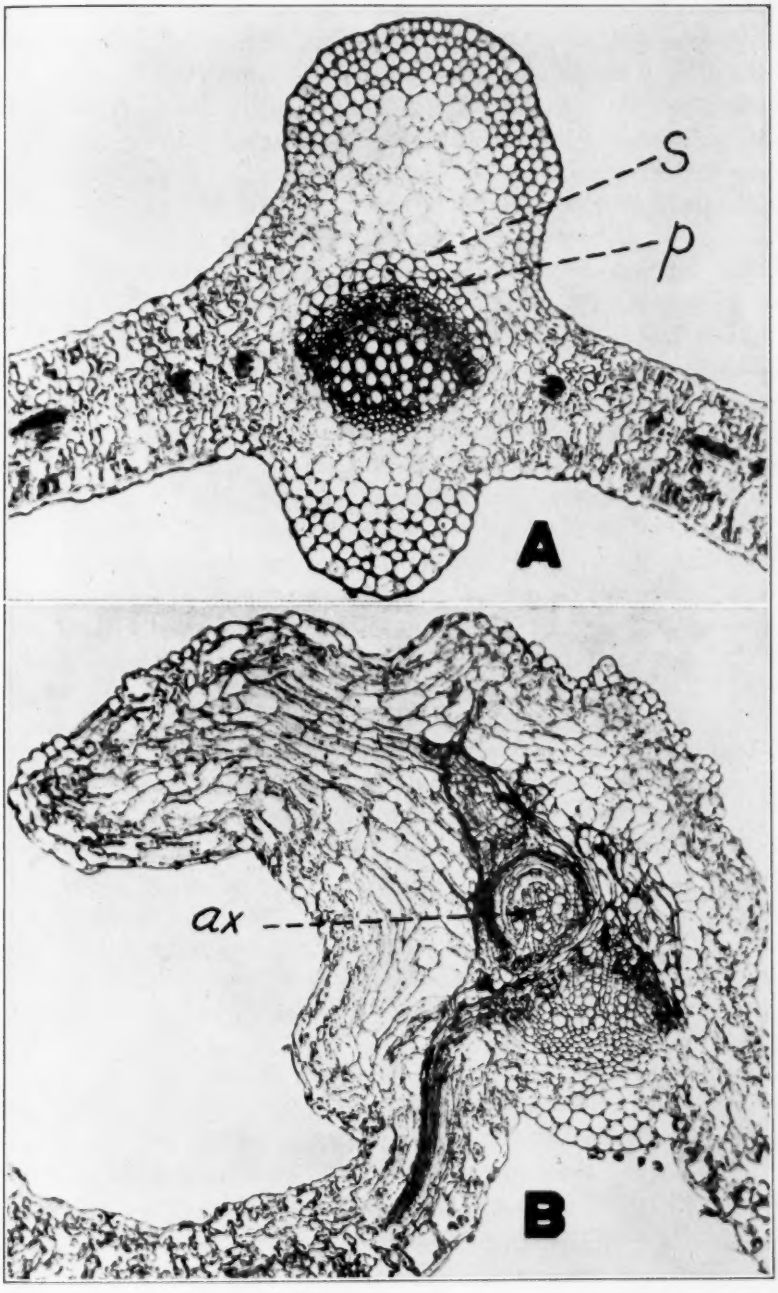

FIG. 10. Transverse sections of lateral veins. ×73. A. Healthy, *p*, pericycle; *s*, starch sheath. B. Diseased, showing proliferation and radial elongation of parenchyma cells external to bundle. Abnormal xylem (*ax*) in bundle cap region.

structures. This fact suggests that the causal factor might be the same in every case (38, 57). Possibly the stimulus to growth and division is furnished by some definite physico-chemical change that may be induced by various means. Smith's (57, p. 511) concept is that this type of change is initiated in injured cells and that all tumors begin in injuries and may be regarded as "excessive and continually modified wound-repair reactions." Gardner (29) has also found that proliferations in tomato-streak mosaic are associated with necroses.

Since the proliferations of tissues along the veins in sugar-beet leaves affected by curly top resemble those observed in other plants, suffering from other types of diseases and injuries, their development might be attributed, not to a specific action of the curly-top virus, but to some cause that is similar in the case of all such structures. Growth and cell division might be stimulated by processes involving changes in the concentration of cell constituents. An accumulation of sugars in curly-top leaves has been shown to occur and was interpreted as the result of disturbed translocation in the degenerating phloem (15). Like the hyperplasias studied by Smith (57) and Gardner (29), the overgrowths in sugar beets are associated with necrotic lesions and the stimuli that lead to formation of the hyperplastic tissue near the lesions and to the proliferations of tissues farther removed from the lesions may perhaps be similar.

DISCUSSION

The disease known as curly top induces, in affected plants, anatomical changes, which involve hypertrophy, hyperplasia, hypoplasia and necrosis. The change last mentioned is limited to the phloem and pericycle. Pathologic effects of similar nature have been observed in many other virus diseases.

The effect of virus diseases is commonly noted throughout the plant. Curly top is such a disorder, necrosis of the phloem occurring in leaves, stems, and roots. Leaves developing after infection usually show symptoms and are stunted; the root is retarded in its growth and produces numerous laterals.

The first sign of degeneration has been observed in the phloem and pericycle and is probably the primary effect of the disease. Cell proliferations in the phloem following necrosis, hypertrophy, and hyperplasia in the mesophyll, and the general stunting of the plant, may be interpreted as secondary effects. These may conceivably be responses to a disturbed nutrition caused by degeneration and necrosis of phloem. If disturbance in the phloem results in sugar accumulation in certain exterior cells, the latter may be stimulated to growth and division, thereby producing proliferations along the veins. Similar nutritional disturbances may cause cell prolifer-

ations adjacent to necrotic lesions. The stunting of the plant undoubtedly results from a deficiency in nutrition, caused by phloem degeneration.

One might suggest, then, that the primary change in the plant, phloem degeneration, is induced by the causal agent of curly top in this tissue; that the secondary changes are, at least in part, responses of the plant to internal injury and necrosis and to the effects of these upon conduction and organic nutrition. At the same time, the alternative hypothesis, that stimulation to growth and division of cells outside the phloem is a direct effect of the virus, cannot be rejected at this stage of investigation. The studies seem to indicate that among the early responses in the phloem to infection are hypertrophy and hyperplasia. Very soon this stimulation spreads to cells outside the phloem. If hypertrophy and hyperplasia in the phloem result from the presence of the virus, the stimulation of mesophyll cells adjacent to the phloem may have the same cause. It is difficult to conceive that the virus would be strictly limited to the phloem in leaves so young that all cells are still predominantly immature. The hypertrophy of cortex cells along the path of the exudate may also be interpreted in 2 ways: stimulation may be caused by increased nutrition or by the presence of the virus in the exudate.

Whether the virus stimulates cells by disturbing their nutrition, or whether it has a more specific action, remains an open question. Neither do we know whether cells proliferating near necrotic lesions in curly-top beets, as well as in other plants, are stimulated solely by nutritional disturbances, or whether specific substances (wound hormones of Haberlandt) are involved. These questions will probably not be answered until we know more about the stimuli underlying the growth and division of cells, whether they be primarily or secondarily meristematic.

Although the changes in the tissues outside the phloem may be caused, in part, by the same stimulus that leads to hypertrophy and hyperplasia in the phloem, the observations strongly indicate that the phloem and pericycle are the first tissues injured and that the effect of the virus is centered in this region. This conclusion seems significant with regard to the localization of the curly-top virus in the affected plant.

According to Küster (38, p. 383), necrosis in plants is often limited to vascular bundles, either because their elements are particularly susceptible to certain injuries, or because, in many cases, they form the channels of distribution for injurious agents.

Studies on the physiology of virus diseases indicate that some viruses probably move mainly through the phloem. Ringing experiments have shown that certain viruses travel through the bark (4, 7); and studies of the aucuba mosaic of the tomato have demonstrated that the virus does

not enter xylem (12, 13). The rates at which certain viruses have been found to travel through plant tissues (54, 63) are comparable to those reported for the translocation of materials in the phloem. Henderson Smith (58) suggested that, because the rate of spread of certain viruses is too high to be explained by movement from cell to cell, and too slow for conveyance with the water stream, this transport is evidently effected through the phloem.

The curly-top virus moves rapidly through plant tissues (54), including phloem necrosis throughout the plant. The suggestion seems justified that the causal factor of curly top is active mainly within the phloem and that disorders occurring outside of this tissue are mostly secondary—the results of inhibited conduction.

SUMMARY

Curly top induces pronounced anatomical changes in affected leaves, involving hypertrophy, hyperplasia, hypoplasia, and necrosis.

Necrosis has been observed only in the primary and secondary phloem and pericycle. It leads to a collapse of cells and formation of lesions.

Hypertrophy and hyperplasia occur in the phloem and pericycle before and after necrotic lesions are formed. Stimulation to growth and division of cells adjacent to lesions resembles wound-healing reactions. Phloem parenchyma, starch sheath, and cortical parenchyma participate in the proliferations near the lesions.

Abnormal xylem frequently differentiates in the proliferated tissue in the phloem region. Within leaves, this xylem is not connected with the normal xylem. Although phloem parenchyma and pericycle in roots and stems usually give rise to secondary cambium, which differentiates into xylem and phloem, in the leaves, such a development was found to occur only at the base of the petioles, whereas the abnormal xylem is produced in petioles, midrib, and lateral veins. The abnormal xylem differs from the secondary xylem, arising in the pericycle and phloem region, in the characteristics of its components: normal xylem has scalariform, scalariform-reticulate, and pitted vessels and pitted fibers; the abnormal xylem has spiral, scalariform, and scalariform-reticulate vessels and no fibers. The abnormal xylem shows very irregular distribution.

Hypertrophy and hyperplasia are not confined to the phloem but occur in cortical tissue also. The mesophyll cells adjacent to the phloem are stimulated to growth and division, becoming closely packed, with no intercellular spaces. The chloroplasts are few, small, and pale in the hyperplastic tissue; they are often of an irregular shape, indicating degeneration. These changes are held responsible for the symptom called clearing of veins.

In later stages hypertrophy and hyperplasia in cortical tissue lead to thickening and distortion of the veins and to a formation of protuberances. The mesophyll farther removed from the phloem may also be in a hypertrophied condition, causing thickening of leaves.

Hypoplasia is manifested mainly in the dwarfing and stunting of the plant.

It is suggested that injury and degeneration of phloem elements probably result from the presence, within the cells, of the causal agent in curly-top infection. Changes occurring in the phloem region after necrosis and those occurring outside the phloem may be interpreted as secondary responses to internal injury and to the resulting necrotic condition in the phloem. On the basis of this interpretation, a hypothesis has been advanced that the virus of curly top is active mainly in the phloem.

DIVISION OF BOTANY, COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA.

LITERATURE CITED

1. ARTSCHWAGER, E. F. Anatomy of the vegetative organs of the sugar beet. Jour. Agr. Res. 33: 143–176. 1926.
2. ————. Development of flowers and seed in the sugar beet. Jour. Agr. Res. 34: 1–25. 1927.
3. BARY, A. DE. Comparative anatomy of the vegetative organs of phanerogams and ferns. English Transl. by F. O. Bower and D. H. Scott. 659 pp. Clarendon Press, Oxford. 1884.
4. BAUR, E. Weitere Mitteilungen über die infektiöse Chlorose der Malvaceen und über einige analoge Erscheinungen bei Ligustrum und Laburnum. Ber. Deut. Bot. Gesells. 24: 416–428. 1906.
5. BEAUVERIE, J. Sur les modes de dégénérescence des chloroplastes, particulièrement dans le parasitisme. Compt. Rend. Acad. Sci. [Paris] 183: 141–143. 1926.
6. ————. Action du parasite sur la résistance du chondriome-plastidome, sa fragilisation et l'altération de la structure cellulaire. Internatl. Cong. Plant Sci. Ithaca, New York. Proceedings 1926, vol. 2, pp. 1299–1311. 1929.
7. BENNETT, C. W. Virus diseases of raspberries. Mich. Agr. Expt. Sta. Tech. Bul. 80. 1927.
8. BLIESENICK, H. Über die Obliteration der Siebröhren. Inaug. Diss. Erlangen. 63 pp. W. Pormetter, Berlin. 1891.
9. BÖNING, K. Die Mosaikkrankheit der Ackerbohne (*Vicia faba* L.) Ein Beitrag zu dem Mosaik der Papilionaceen. Forsch. Gebiet Pflanzenkr. u. Immunität Pflanzenreich 4: 43–111. 1927.
10. BRIOSI, G. Über allgemeines Vorkommen von Stärke in den Siebröhren. Bot. Zeitung 31: 306–314; 322–334; 338–344. 1873.
11. BUTLER, E. J. Some aspects of the morbid anatomy of plants. Ann. Appl. Biol. 17: 175–212. 1930.
12. CALDWELL, J. The physiology of virus diseases in plants. I. The movement of mosaic in the tomato plant. Ann. Appl. Biol. 17: 429–443. 1930.

13. ————. The physiology of virus diseases in plants. II. Further studies on the movement of mosaic in the tomato plant. Ann. Appl. Biol. **18**: 279–298. 1931.
14. CARSNER, E. Attenuation of the virus of sugar beet curly-top. Phytopath. **15**: 745–757. 1926.
15. ————, and C. F. STAHL. Studies on curly-top disease of the sugar beet. Jour. Agr. Res. **28**: 297–320. 1924.
16. COOK, M. T. The effect of some mosaic diseases on cell structure and on the chloroplasts. Jour. Dept. Agr. Porto Rico **14**: 69–101. 1930.
17. ————. The effect of mosaic on cell structure and chloroplasts. Jour. Dept. Agr. Porto Rico **15**: 177–181. 1931.
18. CRAFTS, A. S. Movement of organic materials in plants. Plant Physiology **6**: 1–38. 1931.
19. CRÜGER, H. Einiges über die Gewebsveränderungen bei der Fortpflanzung durch Stecklinge. Bot. Zeitung **18**: 369–374. 1860.
20. DICKSON, B. T. Studies concerning mosaic diseases. Macdonald College Tech. Bul. 2. 1922.
21. DOOLITTLE, S. P. The mosaic disease of cucurbits. U. S. Dept. Agr. Bul. 879. 1920.
22. DUFRÉNOY, J. Les mosaïques du tabac. Office Agric. Rég. du Massif. Central, Clermont-Ferrand, Bul. 9. 1928.
23. ————. Le vacuome de cellules de canne à sucre affectées de mosaïque. Compt. Rend. Soc. Biol. [Paris] **99**: 503–505. 1928.
24. ————La mosaïque de la canne à sucre. Min. Agr. [France], Ann. Epiphyties **14**: 199–210. 1928.
25. ————. Observations sur les modifications pathologiques de la forme des vacuoles des cellules végétales. Min. Agr. [France], Ann. Epiphyties **14**: 227–268. 1929.
26. EAMES, A. J., and L. H. MACDANIELS. An introduction to plant anatomy. 364 pp. McGraw-Hill Book Co., Inc., New York. 1925.
27. ECKERSON, S. H. An organism of tomato mosaic. Bot. Gaz. **81**: 204–209. 1926.
28. FITTING, H., L. JOST, H. SCHENK and G. KARSTEN. Lehrbuch der Botanik für Hochschulen. Begr. v. Strasburger, Noll, Schenk, und Schimper. Ed. 14. 669 pp. Gustav Fischer, Jena. 1919.
29. GARDNER, M. W. Necrosis, hyperplasia, and adhesions in mosaic tomato fruits. Jour. Agr. Res. **30**: 871–888. 1925.
30. GILBERT, A. H. Net-necrosis of Irish potato tubers. Vermont Agr. Expt. Sta. Bul. 289. 1928.
31. GOLDSTEIN, B. A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. Bul. Torrey Bot. Club **53**: 499–599. 1926.
32. HEIN, I. Changes in plastids in variegated plants. Bul. Torrey Bot. Club **53**: 411–418. 1926.
33. HOGGAN, I. A. Cytological studies on virus diseases of solanaceous plants. Jour. Agr. Res. **35**: 651–671. 1927.
34. JOHNSON, E. M. Virus diseases of tobacco in Kentucky. Kentucky Agr. Expt. Sta. Bul. 306. 1930.
35. KUNKEL, L. O. A possible causative agent for the mosaic disease of corn. Bul. Expt. Sta. Hawaii. Sugar Planters' Assoc., Bot. Ser. 3: 44–58. 1921.
36. ————. Studies on aster yellows. Amer. Jour. Bot. **13**: 646–705. 1926.

37. ————. Virus diseases of plants. Chap. IX, pp. 335–364. *In* "Filterable Viruses," edited by T. M. Rivers. 428 pp. Williams and Wilkins Co., Baltimore. 1928.

38. KÜSTER, E. Pathologische Pflanzenanatomie. Ed. 3. 558 pp. Gustav Fischer, Jena. 1925.

39. ————. Pathologie der Pflanzenzelle. Teil 1. Pathologie des Protoplasmas. Protoplasma Monographien. Band 3. 195 pp. Gebrüder Borntraeger, Berlin. 1929.

40. LECOMTE, H. Contribution a l'étude du liber des Angiosperms. Ann. Sci. Nat., Bot. **10**: 193–324. 1889.

41. LYON, H. L. Three major cane diseases: mosaic, sereh, and Fiji disease. Bul. Expt. Sta. Hawaii. Sugar Planters' Assoc., Bot. Ser. **3**: 1–43. 1921.

42. MAHEU, J., and R. COMBES. Sur quelques formations subéro-phellodermiques anormales. Bul. Soc. Bot. France **54**: 429–442. 1907.

43. MUMFORD, E. P. On the curly top disease of the sugar beet: a biochemical and histological study. Ann. Appl. Biol. **17**: 28–35. 1930.

44. PLAKIDAS, A. G. Strawberry xanthosis (yellows), a new insect-borne disease. Jour. Agr. Res. **35**: 1057–1090. 1927.

45. QUANJER, H. M. The methods of classification of plant viruses and an attempt to classify and name potato viroses. Phytopath. **21**: 577–613. 1931.

46. RAND, F. V. Pecan rosette: its histology, cytology, and relation to other chlorotic diseases. U. S. Dept. Agr. Bul. 1038. 1922.

47. RUHLAND, W. Untersuchungen über den Kohlenhydratstoffwechsel von Beta vulgaris (Zuckerrübe). Jahrb. Wiss. Bot. **50**: 200–257. 1911.

48. SALAMAN, R. N. Crinkle "A," an infectious disease of the potato. Proc. Roy. Soc. London, Ser. B. **106**: 50–83. 1930.

49. SALAMAN, R. N., and R. H. LE PELLEY. Para-crinkle: a potato disease of the virus group. Proc. Roy. Soc. London, Ser. B. **106**: 140–174. 1930.

50. SCHANDER, R., and BIELERT. Nekrose und andere Degenerationserscheinungen im Phloem der Kartoffelpflanze. Arb. Biol. Reichsanst. Land u. Fortsw. **15**: 609–670. 1928.

51. SCHILLING, E. Über hypertrophische und hyperplastische Gewebewucherungen an Sprossachsen, verursacht durch Paraffine. Jahrb. Wiss. Bot. **55**: 177–258. 1915.

52. ————. Ein Beitrag zur Physiologie der Verholzung und des Wundreizes. Jahrb. Wiss. Bot. **62**: 528–562. 1923.

53. SEELIGER, D. Untersuchungen über das Dickenwachstum der Zuckerrübe. (*Beta vulgaris* L. var. *rapa* Dum.). Arb. Biol. Reichsanst. Land u. Forstw. **10**: 149–194. 1919.

54. SEVERIN, H. H. P. Curly leaf transmission experiments. Phytopath. **14**: 80–93. 1924. (Abst.) Phytopath. **14**: 123. 1924.

55. ————. Curly top symptoms on the sugar beet. Calif. Agr. Expt. Sta. Bul. 465. 1929.

56. SIMPSON, J. L. S. A short method of clearing plant tissues for anatomical studies. Stain Tech. **4**: 131–132. 1929.

57. SMITH, E. F. An introduction to bacterial diseases of plants. 688 pp. W. B. Saunders Co. Philadelphia and London. 1920.

58. SMITH, J. HENDERSON. Virus diseases in plants. I. Translocation within the plant. II. The amoeboid intracellular inclusions. Biol. Rev. and Biol. Proc. Cambridge Phil. Soc. **5**: 159–170. 1930.

59. SMITH, R. E., and A. BONCQUET. New light on curly top of the sugar beet. Phytopath. 5: 103–107. 1915.
60. SOLEREDER, H. Systematic anatomy of the dicotyledons. English transl. by L. A. Boodle and F. E. Fritsch. Vol. 2. Clarendon Press, Oxford. 1908.
61. SORAUER, P. Handbuch der Pflanzenkrankheiten. Ed. 3. Band 1. Die nichtparasitären Krankheiten. Paul Parey, Berlin. 1909.
62. SOROKIN, H. Phenomena associated with the destruction of the chloroplasts in tomato mosaic. Phytopath. 17: 363–379. 1927.
63. STOREY, H. H Transmission studies of maize streak disease. Ann. Appl. Biol. 15: 1–25. 1928.
64. STRASBURGER, E. Über den Bau die Verrichtungen der Leitungsbahnen in den Pflanzen. Histologische Beiträge, vol. 3. 1000 pp. Gustav Fischer, Jena. 1891.
65. TOWNSEND, C. O. Curly top, a disease of the sugar beet. U. S. Dept. Agr. Bur. Plant Indus. Bul. 122: 1908.
66. TUNMANN, O. Pflanzenmikrochemie. 631 pp. Gebrüder Borntraeger, Berlin. 1913.
67. VALLEAU, W. D., and E. M. JOHNSON. Some possible causes of streak in tomatoes. Phytopath. 20: 831–839. 1930.
68. VANTERPOOL, T. C. Streak or winter blight of tomato in Quebec. Phytopath. 16: 311–331. 1926.
69. VÖCHTING, H. Über Transplantation am Pflanzenkörper. 162 pp. H. Laupp, Tübingen. 1892.
70. VRIES, H. DE. Beiträge zur speciellen Physiologie landwirtschaftlicher Culturpflanzen. VIII. Wachsthumsgeschichte der Zuckerrübe. Landw. Jahrb. 8: 417–498. 1879.
71. ZIRKLE, C. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science, n.s., 71: 103–104. 1930.

CULTURE OF PHYTOPHTHORA INFESTANS

WILLARD CROSIER

(Accepted for publication Sept. 22, 1932)

Phytophthora infestans (Mont.) de By., pathogen of the common late blight of potatoes, is often desired for experimentation and for class demonstrations in plant pathology and in mycology. It is frequently the case, however, that considerable difficulty is encountered in culturing the fungus. Ordinary culture media, such as potato-dextrose agar, oatmeal agar and cornmeal agar, which are useful in growing other species of Phytophthora, support only a feeble growth of *P. infestans*. A number of workers, including Clinton (2), Jones *et al.* (3), Rosenbaum (6), and Tucker (7), comment on the extremely meager growth of *P. infestans* on vegetable and cereal agars and on synthetic media. It is not impossible to grow the fungus on oatmeal agar but the resultant development of mycelium and sporangia necessitates the use of an unwieldly number of test tubes, Petri dishes, or flasks.

The possibility of culturing the fungus on oatmeal agar as a means of maintenance only and its transfer to potato tubers or foliage to develop large numbers of sporangia or to demonstrate the pathogen-suscept relationship has been suggested. It has been found that mycelium transferred to tubers in some cases causes a tuber rot but in the majority of cases does not. Practically all of the students who have attempted to develop a tuber rot by this method have been unsuccessful.

The best method of growing the fungus in pure culture is that of using raw aseptic tuber plugs as suggested by Jones *et al.* (3) and as improved by Löhnis (4). This method consists of surface sterilizing tubers and removing plugs of the inner tissue with a sterilized cork borer. The plugs are then placed in sterilized test tubes containing a small amount of water. This method has not been very popular since it necessitates very careful work in the preparation of the plugs, and the surface available for the formation of the aerial mycelium and the sporangia is not large enough to supply sporangia in quantity.

In a recent investigation dealing with sporangium production and germination it was necessary to develop sporangia in abundance at will and to be able to remove them for use while they were still viable. The method mentioned above was not satisfactory. It has been found much better to grow the fungus continuously on either disinfected tuber slices or on potato foliage. Transfers are made as often as necessary to maintain a thrifty clean growth. When plants of a susceptible variety are available they are inoculated with either sporangia or preferably with swarm spores. If only

a few sporangia are desired a few leaflets may be inoculated, or if a large number of sporangia are needed for extensive study or for demonstrations several plants may be thoroughly inoculated. The spores are either sprayed on with an atomizer, dropped on from a pipette or sprinkled on with a common clothes sprinkler. At an incubation temperature of 20° C. lesions appear in 3 to 5 days. The diseased leaves are then detached and placed in a moist chamber to permit sporulation. Sporangia form in abundance over the entire necrotic area and in the surrounding margin of green tissue in 8 hours or more, depending on the temperature. When it is desired to maintain the fungus for an indefinite period with a minimum of attention the plants are placed at a temperature as low as 10° C., if possible. The fungus grows slowly at this temperature, so that the plants are not killed for several weeks. When sporangia are needed the plants are placed in a moist chamber at 18 to 20° C. Under these conditions the fungus rapidly extends throughout the host plant and sporulates readily and abundantly.

When it is inconvenient or impossible to employ plants for substrata, thin slices cut from washed and surface-sterilized tubers may be utilized. Sound tubers are washed in warm water, dipped in $HgCl_2$ (1 to 1000) for 5 minutes or longer, rinsed in sterilized water and cut into slices with a sterilized scalpel. Under ordinary conditions it is necessary only to wash the tubers, dip them in 70 per cent alcohol, and flame them to insure a moderately clean and sterile surface. The slices are immediately placed in clean Petri dishes containing moist filter paper.

The fungus may be transferred to the slices by diseased tissues, aerial mycelium, sporangia or swarm spores. Either of the first two may be stabbed into the upper surface of the slice with a flamed scalpel. In case a suspension of sporangia or of swarm spores is used for the inoculum a small depression or well is made in the slice and a drop of the suspension placed therein. This permits the fungus and also the bacteria to radiate from a common focus, and the more rapidly growing fungus is enabled to advance practically free of inhibiting bacteria. The Petri dishes are then covered for at least 2 hours to maintain that high relative humidity essential for the growth of the germ tubes and for their penetration into the new substratum. Establishment of the fungus proceeds most rapidly at temperatures of 9 to 24° C., with an optimum at 19 to 22° C.

The further treatment of the slices depends on whether a new growth is desired in the briefest possible time or only after a longer period. The optimum conditions for incubation are: a temperature of 19 to 21° C. and a relative humidity of approximately 100 per cent. Under these conditions aerial mycelium appears on the slices within $2\frac{1}{2}$ to 3 days, depending somewhat on the variety used for the substratum. The development of aerial mycelium and, therefore, sporangia, is retarded by a slight decrease in the

relative humidity and is entirely prevented when the latter is below 90 per cent. Subjecting the slices to low relative humidities results in decreasing the aerial mycelium and, what is more important, retards the growth of bacteria. It has been found that infected tubers or tuber slices exposed to ordinary room temperatures and humidities will not rot entirely for several weeks and that when afterwards placed in a moist chamber will give rise to sporangia in abundance. Bacterial contamination also is decreased or entirely eliminated by incubating the slices at temperatures below the optimum (19 to 21° C.) for growth of the fungus. Cultures have been held at 6° C. for as long as 30 days with no appreciable amount of contamination due to bacteria. The initiation of sporulation is considerably delayed and its continuation favored by the lower temperatures. In a number of experiments slices were placed at 6° C. within a few hours following transfer of spores and examined daily. Aerial mycelium only rarely formed within 15 days and sporangia usually appeared in about 20 days. This indicates that the fungus may be maintained with very little attention by growing it at a low temperature. At an incubation temperature of 9° C. mycelium appeared in 12 to 14 days, at 12° C. in 6 to 7 days and at 15° C. in 4 to 6 days.

A lot of Rural Russett tubers, artificially inoculated with *Phytophthora infestans*, were held from October, 1931, to July, 1932, at a temperature of 4 to 6° C. and a relative humidity of 80 to 85 per cent. Very little bacterial rot was evident at the termination of this period, although some of the tubers were nearly destroyed by Phytophthora rot. The fungus was induced to grow vigorously and to sporulate copiously simply by placing the tubers in a saturated atmosphere at 18° C. Fructifications formed in abundance in 24 hours or less. More or less similar conditions exist in ordinary storage of tubers over the winter months and similar overwintering of the fungus can be considered an actuality.

It has been shown that the production of sporangia of *Phytophthora infestans* is of common occurrence when the atmosphere is saturated with water vapor and takes place over a temperature range of 6 to 24° C. The results of several experiments show that sporulation occurs at a minimum temperature of 3° C. and at a maximum of 26° C. Vowinckel (8), working in Germany, reports essentially identical results. Only a few of the sporangia that form throughout this range of temperatures will germinate either by germ tubes or by swarm spores unless precautions be taken to maintain their viability and to supply the correct conditions for their germination. *P. infestans* in common with most species of the order Peronosporales forms aerial sporangia only when moisture is present and these sporangia remain viable and germinate only in the presence of moisture or, at least, a very high relative humidity.

Under optimum conditions the sporangia remain viable for only a few days. A high relative humidity is absolutely essential for maintenance of viability, and low temperatures prolong the period of viability. Melhus (5) observes that sporangia, held for 6 hours in a dry laboratory, are not able to germinate; while Löhnis (4) reports that no sporangia survive an hour of rapid drying. Other workers state that low humidities and high temperatures adversely affect the longevity of the sporangia, but data based on even approximately controlled factors have not been available until the last few years. Vowinckel (8) attempted to find the relative humidities necessary for sporangium formation and longevity but was not very successful because of poor control of humidity.

In a series of experiments in which the relative humidity has been controlled to within 1½ per cent it has been found that the lower the relative humidity the more rapid the loss of viability. Lots of sporangia that gave 80 per cent indirect germination at 13° C., as soon as mature, have been held at a temperature of 20° C. and various relative humidities. Sporangia exposed to 99 per cent relative humidity for an hour gave 72 per cent indirect germination; those held at 90 per cent for an hour gave 45 per cent germination; those at 50 per cent gave 11 per cent germination; and those held at 25 per cent gave only 5 per cent indirect germination. Similar lots of sporangia have been exposed to temperatures of 25 and 30° C. and various relative humidities. In these cases the loss of viability was even more pronounced. As a comparison, sporangia exposed to 25° C. and 90 per cent relative humidity for an hour gave 40 per cent indirect germination, whereas those held at a similar relative humidity and at a temperature of 30° C. gave only 32 per cent. indirect germination. Reduced humidities and high temperatures affect the ability of the sporangia to germinate directly, although to a lesser degree.

The experiments of Melhus (5) demonstrate that a greater percentage of sporangia will germinate by swarm spores at 13° C. than at any other temperature to which he exposed them. The optimum temperature for germ-tube formation is reported to be 24° C. It has been found in similar experiments that sporangia of *Phytophthora infestans,* regardless of their age or the conditions under which they have been stored, germinate most rapidly and abundantly at 12 to 13° C. by swarm spores and at 24° C. by germ tubes. The range of indirect germination extends from 1° C. or lower to about 25° C. and that of direct germination from 6° C. to nearly 30° C. The curves in figure 1, A, show the relation of temperatures from 1½ to 30° C. to both indirect and direct germination of variously treated sporangia. A considerably greater percentage of sporangia germinate by swarm spores (curves IM, IO, IE) than by germ-tube formation (curves M, O) at temperatures of 1½ to 18° C., whereas the opposite is true at temperatures

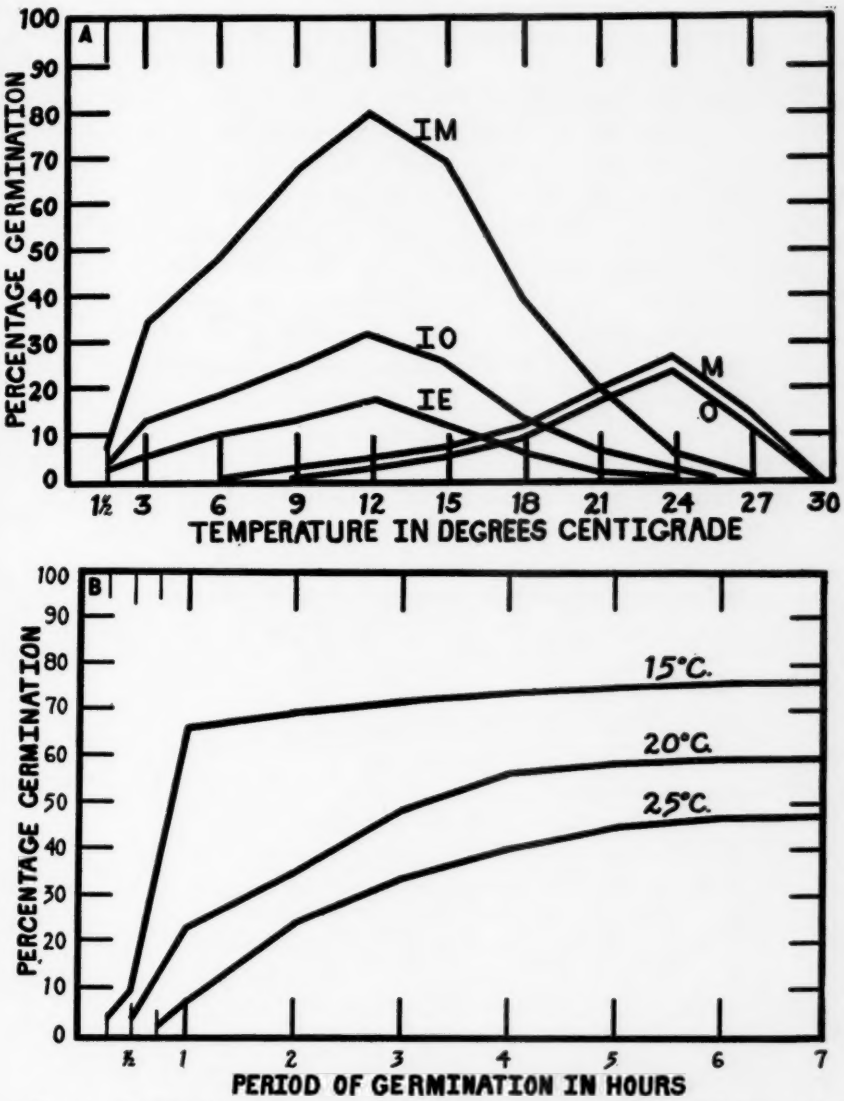

FIG. 1. Germination of sporangia of *Phytopthora infestans*. A. Relation of temperture to indirect germination (IM, IO, and IE) and to direct germination (M and O). The curves are based on the actual percentages of sporangia that had germinated after exposure for 50 hours to the indicated temperatures. B. Rapidity of indirect germination at 13° C. Sporangia were removed from cultures that had been incubated at 15, 20, and 25° C.

of 21 to 30° C. The relation of age and storage conditions of the sporangia to indirect germination is shown by curves IM, IO, IE. Old sporangia, curve IO, and sporangia that have been exposed a few hours to a dry atmosphere, curve IE, germinate less rapidly and also less abundantly than freshly matured sporangia, curve IM. It is evident from curves M (mature sporangia) and O (old sporangia) that the same relation of age to percentage of germination exists in direct germination of the sporangia, although it is by no means so marked as in indirect germination.

The rapidity of indirect germination of sporangia immersed in water and exposed to the optimum temperature of 13° C. is shown in figure 1, B. The influence of the temperature under which the sporangia have developed and have been stored is shown by the three curves. The most rapid and abundant germination occurred in these lots of sporangia that had formed on slices and leaves incubated at 15° C. Sporangia of the same age, formed and held at 20 or 25° C. until placed under conditions requisite for germination, germinated less rapidly and less abundantly. This indicates, as field observations have confirmed, that, even though the relative humidity approach 100 per cent, sporangia that are exposed to temperatures of 25° C. or higher for a few hours germinate very poorly, if at all, whereas those subjected to a temperature not exceeding 15° C. remain viable for several hours. No germination of sporangia has ever been observed unless water be present in the liquid state. The existence of a high relative humidity of 98 to 100 per cent does not suffice; the sporangia must be in contact with a film of water. Ordinarily, a slightly better germination occurs in a shallow than in a deep receptacle, and the results of many experiments show the presence of vaseline, bentonite clay, or finely dispersed infusorial earth increases the percentage of indirect germination.

If one wishes to demonstrate the phenomenon of swarming, the swarmspore suspension should be held at a temperature as near as possible to 3° C. The duration of motility decreases from 22 hours at 3° C. to 30 minutes at 24° C. A culture of swarm spores thus can be held for several hours in a motile condition simply by placing them at a low temperature. It also has been found advantageous in inoculation experiments to place the swarmspore suspension at 3° C. if plants are not ready for inoculation as soon as the swarm spores have formed. The swarm spores, after coming to rest, germinate readily at 12 to 24° C. and less readily at 3 to 9° C. The optimum temperature in relation to percentage of swarm spores that germinate is 15° C., while the germ tubes elongate most rapidly at 21° C. and die quickly at 26° C. or above. In inoculation work the foliage of the plants should be kept moist for at least 1½ hours if the temperature is around 20 to 25° C., 2 hours if 15° C., and 2½ to 3 hours if 10° C. The

percentage of penetration of germ tubes, however, continues to increase for as long as 8 hours at 10 to 25° C. After this period there is no particular advantage in keeping the plants in a moist atmosphere. Numerous inoculation experiments have been made using a swarm-spore suspension containing 10 spores to a drop. At 10° C. only 4 per cent of the inoculations resulted in infections when the plants remained moist for 3 hours, but 85 to 90 per cent of the inoculations were successful when the plants were kept in the moist chamber for 8 hours. Comparable percentages for 15° C. are 9 and 88, and for 21° C. are 13 and 91.

As previously mentioned, the optimum temperature for direct germination of the sporangia (conidia) is 24° C. The process of germ-tube formation is, compared to formation of swarm spores, relatively slow and rarely more than 20 per cent of the sporangia germinate directly. In only one case have germ tubes been observed until the sporangia had been at the optimum temperature of 24° C. for over 4 hours. Ordinarily some germination occurs in 8 hours at 18 to 26° C., although some lots of sporangia held at 24° C. have not started to form germ tubes until the third day. It is possible, as Blackwell and Waterhouse (1) mention, that maturation of the sporangia is a factor in the percentage and rapidity of direct germination. From the facts that more sporangia germinate indirectly than directly, that one sporangium may form 8 to 20 swarm spores but only one germ tube, and that the process of liberation of swarm spores and their subsequent germination consume considerably less time than the formation of germ tubes directly from the sporangia, it is to be expected, as many experiments have shown, that *Phytophthora infestans* spreads rapidly in the field when the temperature favors indirect germination of the sporangia but very slowly when the temperature favors direct germination.

SUMMARY

Suggestions are given for culturing *Phytophthora infestans* in pure culture and on potato tubers and foliage.

Bacterial contamination is eliminated by incubating the tuber cultures at low temperatures or at low relative humidities.

Sporulation occurs over a temperature range of 3 to 26° C. A saturated atmosphere is essential for sporangium formation.

A high relative humidity is necessary to preserve viability of the sporangia. The period of viability also is increased by holding the sporangia at low temperatures.

The optimum temperature for indirect germination of the sporangia is 12 to 13° C., and for direct germination is 24° C.

The sporangia of *P. infestans* germinate only in the presence of water. Bentonite clay and certain other substances increase the percentage of indirect germination.

Swarm spores of *P. infestans* may be maintained in a motile condition by holding the spore suspension at 3° C.

A small percentage of the swarm-spore germ tubes penetrate the host tissue in 1½ to 3 hours. Penetration proceeds most rapidly at 20 to 25° C.

The results of many experiments tend to prove that *Phytophthora infestans* spreads most rapidly in the field when the temperature favors indirect germination of the sporangia.

CORNELL UNIVERSITY,
ITHACA, N. Y.

LITERATURE CITED

1. BLACKWELL, E. M., and G. M. WATERHOUSE. Spores and spore germination in the genus *Phytophthora*. Brit. Mycol. Soc. Trans. **15**: 294–310. 1931.
2. CLINTON, G. P. Oospores of potato blight, *Phytophthora infestans*. *In* Report of the botanist Connecticut Agr. Expt. Sta. Rpt. **1909–1910**: 753–774. 1911.
3. JONES, L. R., N. J. GIDDINGS, and B. F. LUTMAN. Investigation of the potato fungus *Phytophthora infestans*. U. S. Dept. Agr. Bur. Plant Ind. Bul. 245. 1912.
4. LÖHNIS, MARIA PETRONELLA. Onderzoek over *Phytophthora infestans* (Mont.) de By. op de aardappelplant. Proefschrift Univ. Utrecht. 96 pp. H. Veenman, Wageningen. 1922.
5. MELHUS, I. E. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*). Wisconsin Agr. Expt. Sta. Res. Bul. 37. 1915.
6. ROSENBAUM, J. Studies of the genus *Phytophthora*. Jour. Agr. Res. **8**: 223–276. 1917.
7. TUCKER, C. M. Taxonomy of the genus *Phytophthora* de Bary. Missouri Agr. Expt. Sta. Res. Bul. 153. 1931.
8. VOWINCKEL, OTTO. Die Anfälligkeit deutscher Kartoffelsorten gegenüber *Phytophthora infestans*. (Mont.) de By., unter besonderer Berücksichtigung der Untersuchungsmethoden. Arb. Biol. Reichsanst. Land. u. Fortsw. **14**: 588–641. 1926.

ASCOSPORE DISCHARGE IN OPHIOBOLUS GRAMINIS, AND ITS PROBABLE RELATION TO THE DEVELOPMENT OF WHITEHEADS IN WHEAT

GEOFFREY SAMUEL AND S. D. GARRETT

(Accepted for publication January 4, 1933)

The epidemiology of the take-all disease of wheat, caused by the fungus *Ophiobolus graminis* Sacc., is comparatively little understood as yet. There have been two main suggestions as to the method of dissemination of the fungus in the field: (1) that "ascospores constitute the inoculum for the vast majority of infections" (Kirby (3)) and (2) that mycelium, carried by the wind with soil particles and in pieces of tissue from the bases of infected cereals and grasses, forms an important additional method of spread (McAlpine *et al.* (4)).

A number of workers have studied ascospore discharge, and most agree that when ripe perithecia are placed in water the asci are ejected singly into the water, where they quickly burst, liberating the spores. Waters (5), however, figures asci and ascospores being extruded in a tendril through the ostiole.

Kirby (3) stated "it is clear that the behavior of the ascospores at the time of their discharge restricts the agents of dissemination almost completely to rain." He also considered that the delicate nature of the ascospores rendered them unsuitable for dissemination by wind, and he determined that spores from a drop of water, allowed to dry on a glass slide, lost their power of germination within half an hour.

The two statements of Kirby, quoted above, seem entirely inadequate to explain the known field occurrence of take-all. If ascospores are the main source of infection and if they can be disseminated only by rain washing them about in the soil, the appearance of take-all in the whitehead stage throughout 100-acre fields of wheat sown on fallow, as frequently occurs in Australia in seasons favorable to the disease, would be impossible to explain.

We have recently made the observation that during periods of rain ascospores may be ejected from the perithecia into the air at the rate of several hundred per minute from a single diseased wheat culm, in much the same manner that the ascospores of *Venturia inaequalis* are ejected during rainy periods in spring from dead apple leaves on the ground. In addition to this, fields badly infected with whiteheads were found to have a number of small patches in them in which the plants had become diseased in the early stages in the typical take-all manner and on which perithecia were present. It is considered that these two facts, combined, throw new light on the epidemiology of take-all.

INCIDENCE OF TAKE-ALL IN SOUTH AUSTRALIA

The incidence of take-all in the wheat fields of South Australia may be described briefly as follows: Crops sown on new land just cleared from Eucalyptus scrub are usually entirely free from the disease. It is the practice to take 2 or 3 wheat crops in succession off such land, and in the second and third years isolated plants or small patches affected with take-all can often be found and occasionally there is a quite serious infection. After this period, depending on the weather, the growing of further wheat crops may become a gamble unless oats or bare fallow is brought into the rotation. Take-all definitely becomes the limiting factor to the frequency of cropping with wheat. As time goes on and farms are brought into full cultivation, with the adoption of good crop rotations, methods for better consolidation of the soil, improved manurial practices, etc., take-all gradually becomes comparatively rare unless induced by special circumstances. The older wheat-growing districts are, therefore, frequently spoken of as having "passed through the take-all phase," and the disease is no longer feared.

EPIDEMICS

Epidemics of take-all appear to result in this country from the coincidence of 3 main factors: (1) lack of consolidation of the soil, (2) the presence of some inoculum in the soil, and (3) last, but not least, the occurrence of showery weather lasting almost until the crop is in head.

By far the greatest loss from take-all is due to the development of the whitehead stage of the disease just before the crop is ripening. Splendid crops, 4 ft. or more in height, in which only a comparatively few take-all patches were evident before flowering, may suddenly develop whiteheads after flowering, so that more than 80 per cent of the grain is lost and nothing can be done but reap the crop for a poor-quality hay, or burn it. Widespread losses of this type occur only in years when the ripening weather is showery and only on soils not well consolidated. The serious nature of the disease in seasons favorable to it is evident from the fact that in the State of South Australia, alone, take-all took in 1932 a toll of several million bushels of wheat.

The 3 factors mentioned above as determining the occurrence of serious epidemics of take-all may be discussed in somewhat further detail as follows:

(1) *Consolidation of the Soil.* Most of the affected crops are on land of a light, sandy texture. Moreover, instances frequently occur of a crop on light land remaining healthy only in parts where the soil has been well compacted. The following instance, given by a farmer on Eyre Peninsula, is fairly typical: "The paddock fallowed was 170 acres and sheep had access to it the whole season, a gate being left open between the fallow and

a grass paddock containing water. About 50 acres in the vicinity of the gate, and some 10 acres on a rise where the sheep made a camp, were so solidly compacted by the sheep that the wheat on these portions should yield 24 bushels to the acre. The crop is healthy, dense, and of good even colour, while on all other parts of the fallow take-all is very severe."

In the effort to get maximum consolidation of the lighter soils of the mallee wheat-growing areas, the best farmers plow only about 2 in. deep and keep their fallow lands clean either with sheep or with tined cultivators working to a shallow depth (1).

Take-all, however, can occur also on heavier land. In such cases it usually has been induced either by too deep plowing or by plowing in pasture grasses and cropping the following year, especially when seasonal conditions have not allowed a proper compacting of the soil. The deepest-plowed plot (12 in.) in the depth-of-plowing tests at Roseworthy Agricultural College is always the plot most severely affected by take-all.

It cannot yet be said whether the greater severity of take-all on soils that are not well consolidated is due to the wheat plants being less resistant to the disease under such conditions, or to there being more favorable conditions for the activity of the fungus in loose soils, or to both of these factors acting together.

(2) *Inoculum in the Soil.* Very little is known about the distribution and concentration of take-all inoculum in the soil. Since, in a favorable year for take-all, a field in which the soil is poorly consolidated will practically always suffer severely from the disease, the opinion seems to be current in Australia that the take-all fungus is present almost everywhere in the soil, awaiting only favorable conditions to become an active parasite. But, there are several facts that would be very difficult to explain on this hypothesis. Why should a few take-all patches show up early in the season and kill the plants in the seedling stage, whereas by far the greatest loss does not appear until after flowering? The plants that succumb to white-heads could scarcely have been growing in contact with *Ophiobolus graminis* since seeding time, and yet no method of distribution of the fungus over large areas during the growing period of the crop has yet been suggested.

The question of the amount of inoculum in the soil will be discussed in greater detail by one of us at a later date. No doubt there are circumstances when take-all inoculum might be fairly generally distributed in a soil at seeding time. This might occur, for instance, if a grass paddock in which a considerable amount of barley grass was infected with take-all was plowed late, especially if the plowing was followed by dry weather. But, the suggestion made here is that in most cases the soil of the fallows is largely free from inoculum and that the positions where inoculum exists at seeding time are the comparatively few places where take-all patches

develop in the crop in the seedling stage. Whether these "primary patches" are due to ascospore infections or to pieces of infected stubble, remaining in the fallow or being blown onto it in summer, will not be further considered here.

(3) *Weather Previous to Ripening.* It has been stated above that serious epidemics of take-all occur only in years when showery weather lasts almost until the time of ripening of the crop. Conversely, when the spring is fine and dry, comparatively little take-all in the whitehead stage can be found.

The relation between the amount of spring rain and take-all may be seen in table 1. The explanation now proposed for this relation is that ascospores, maturing in perithecia on the dead plants in the primary take-all patches, are dispersed over the field in wet spring weather, giving rise to a scattered secondary infection, manifested in the development of whiteheads.

There are no doubt other factors than spring rainfall of which account must be taken. For instance, take-all was much more severe in 1932 than in 1930, although the amount of spring rain was actually somewhat less. A possible explanation may be advanced as follows: The late rains in 1930 were no doubt favorable to infection of grass paddocks, as well as crops, and many of these grass paddocks were plowed up for fallowing about August, 1931. The subsequent dry spring and summer would have allowed more of the fungus to survive as viable initial inoculum for the 1932 patches. The rains in 1932 came early and led to earlier sowing of wheat; this may have given, by spring, an opportunity for the development of more perithecia than usual. When the spring of 1932 also happened to be wet everything was apparently favorable for the severe epidemic of take-all that actually occurred.

TABLE 1.—*The approximate severity of take-all on poorly consolidated soils in the mallee wheat-growing districts of South Australia since 1921, together with the September–October rainfall at Adelaide*

Year	Sept.–Oct. rain	Take-all	Year	Sept.–Oct. rain	Take-all
1921	4.9	Severe	1927	1.4	Light
1922	3.3	[a]	1928	4.2	[a]
1923	8.1	Severe	1929	3.2	Light
1924	5.5	Severe	1930	5.4	Fairly severe
1925	3.3	[a]	1931	3.3	Light
1926	4.7	[a]	1932	4.3	Severe

[a] No definite reports available, but the disease could have been only light to moderate or it would have been definitely noted.

SPORE DISSEMINATION

The method of ascospore discharge from the perithecia was discovered in the laboratory. Specimens of diseased wheat plants had been obtained from take-all patches in a crop of wheat some 5 ft. high, which was rapidly going off with whiteheads. There were only a few of these early diseased patches, but the plants in them had died when only about a foot high, and a number of perithecia were present on the bases of the culms. Small pieces of leaf sheath, containing perithecia, were cut out, laid on glass slides, and allowed to soak up drops of water until fully moistened. Clean cover glasses were suspended about a millimeter above them, resting on 2 matches. The preparations were kept under observation through a binocular microscope.

Spore ejection commenced in most cases some 10 to 30 minutes after moistening. Directly above the protruding neck of each ripe perithecium the spores were observed hitting the under surface of the cover glass in groups of 8 as each ascus was extruded and burst. The 8 spores hit the glass simultaneously and adhered to it. Single perithecia ejected asci at a rate of from 2 to 10 per minute, when in good condition, the rate gradually slackening and ceasing entirely after an hour or 2. A piece of tissue about $\frac{1}{8}$-inch square with 6 perithecia on it ejected spores at the rate of considerably more than 100 per minute for over an hour. Fresh cover slips were put on every minute and were then placed in Petri dishes for the longevity tests described below.

The same type of spore ejection was proved to occur in the field when perithecia-bearing culms were moistened with rain water. By holding a sticky glass plate behind them and gently blowing, it was also shown that the ascospores could be carried away in air currents as they were ejected.

Since the asci in the perithecia of *Ophiobolus graminis* are not all ripe at once, it is probable that ascospore discharge takes place from the same perithecia on many successive occasions during a rainy spring. Spore ejection has been obtained from small pieces of tissue in the laboratory on several successive days.

Occasionally, perhaps when the perithecia are not fully moist, the forcible projection of the ascospores as the ascus comes to the mouth of the perithecium fails to take place. There is then a gradual accumulation of asci at the ostiole, but nothing so marked as some of the coiled tendrils figured by Waters (5) has been seen.

LONGEVITY OF THE SPORES

The longevity of the spores was tested by germinating them in bean decoction at 24° C. at intervals of 12 hours following their ejection from perithecia to sterile cover slips. In each experiment all of the spores used

came from the same group of discharging perithecia. The cover slips, each with some 50 to 100 spores, were divided between 4 sterile Petri dishes. Two were kept at a temperature of 12° C. and 2 at a temperature of 24° C. Of the 2 at each temperature 1 was dry, and the other had a piece of moist filter paper inside the lid. The results of 1 series of germination tests are given in table 2. There was some variation between different batches of material, but table 2 gives results typical of good material.

TABLE 2.—*Results of germination tests at 12-hour or daily intervals on ascospores of Ophiobolus graminis. The spores were ejected onto sterile cover slips and were kept at 2 different temperatures and 2 different humidities*

Cover slips kept in incubator at	Time after ejection									
	0	12	24	36	48	60	72 hours	4	5	6 days
24° C. dry	+	+	+	+	+	+	+	−	−	
moist	+	+	+	+	+	+	+	+	+	+
12° C. dry	+	+	+	+	+	+	+	+	−	−
moist[a]	+	+	+	+	+	+	+	+	+	+

[a] Drops of moisture condensed on the cover glasses in the moist dishes at 12° C. after about 2 days, and a few of the spores germinated; these were excluded from the count when the germination test was done.

The average air temperature in the field during rainy periods in spring would not be much above 12° C.; and, since the humidity would be high, the spores probably would be viable for several days under field conditions. This would give ample opportunity for wind dissemination and for infection in a rain-sodden soil.

DISCUSSION

A number of points about the occurrence of take-all, hitherto very difficult to explain, become much easier to understand if aerial dissemination of ascospores takes place. There is not only the occurrence of early and late infection but the fact that the former is always in patches and the latter usually scattered; the relation between spring rains and the development of serious epidemics of whiteheads; the abundant infection that sometimes occurs on fallowed paddocks; the appreciable infection occasionally recorded on new land, etc.

With regard to the longevity of the spores in its relation to aerial dissemination, our method of collecting naturally ejected ascospores by suspending cover glasses close to the necks of perithecia is a much preferable way of obtaining material for longevity tests than Kirby's method of allowing spores in a drop of water to dry. A longevity of several days, for dry spores, seems a much more reasonable result than Kirby's half-hour, and would give ample opportunity for the dissemination of the fungus over considerable distances in rainy weather.

Air-filtration experiments in the field, such as those carried out by Keitt and Jones (2) for *Venturia inaequalis,* would probably lead to similar results for the take-all fungus. That is, in fine weather few spores or none are likely to be found in the air, but soon after rain falls the spore concentration probably rises considerably. In the case of take-all, however, instead of the spores infecting the aerial parts of plants, as in apple scab, they are with little doubt soon carried down in the rain over the large areas of wheat fields and infect through the roots. Further studies of root infection by ascospores will, therefore, be of much interest.

The explanation here advanced for the development of epidemics of the whitehead stage of take-all certainly does not present a complete picture of the epidemiology of the disease. In the epidemic season of 1932, in the same district in which could be found instances of severe crop losses most readily explainable on the above hypothesis, there were also to be found cases where primary take-all patches had appeared in crops and no secondary spread had occurred. This may have been due, however, to the fact that perithecia did not mature early enough on the different varieties of wheat involved, or to a difference in soil character, which rendered the wheat plants nonsusceptible at the time of ascospore discharge, or most probably to soil conditions unfavorable to infection by ascospores. Cases also were seen of a moderate (perhaps 5%) scattered whitehead infection through crops that could not be definitely traced to any particular primary patches. Diseased barley grass, bearing perithecia, was, however, found in the vicinity, and, although the infections did not seem to be markedly more numerous near the barley grass, this offers a possible explanation. There is no doubt that the field occurrence of take-all is influenced by many interacting factors (soil, climatic, and biological) and that much more information is needed concerning all of them.

Finally, with regard to the bearing of these facts on practice, some fresh problems are raised. Would the digging under of the early take-all patches be an effective control measure against the extensive whitehead infection that develops on certain soils in wet springs? At present all the emphasis is laid on elimination of the source of infection (apparently considered as extensively distributed in the soil) by means of crop rotation, combined with thorough consolidation of the soil. Both these methods are of the greatest practical value, and it is desirable that primary emphasis should continue to be laid on them. But, in a favorable season for take-all, there are often heavy losses from the disease on land that has been reasonably well-fallowed. And there are also light sandy soils in the mallee districts of Australia that cannot yet be properly consolidated by any treatment. Under these circumstances it would appear well worth while to test the third possibility of control, namely, destroying the plants in the primary

take-all patches to prevent the formation of perithecia on the dead plants, and thus eliminate what now appears to be the most likely source of secondary infection. This method, of course, would be impracticable where primary infection was widespread.

There is one fact that might render such action much less efficacious than would appear at first sight and that is that much of the barley grass, naturally present along the fences and in pasture paddocks, is often infected with take-all. It is possible that these plants may provide a source of ascospores for wind dissemination. At present the evidence would suggest that if they do so it is only to a very limited degree compared with the wheat plants in the primary take-all patches in the crop; but an answer to these and other problems will have to await further work in the field.

SUMMARY

It is shown that moistened perithecia of *Ophiobolus graminis* eject ascospores into the air. The rate of discharge may attain several hundred spores per minute from a single diseased wheat culm. The ascospores may be carried by the wind, and their longevity is in the neighborhood of from 3 to 4 days under dry conditions, and as much as a week under moist conditions, at a temperature of 12° C.

The greatest loss from take-all in South Australia occurs in the whitehead stage and in seasons when showery weather lasts almost until the crop is in head. In crops rapidly going off with a scattered whitehead infection, early diseased take-all patches were always found in which the plants had died before flowering and on which perithecia were present. Taken in conjunction with the method of ascospore discharge described above, this suggests that epidemics of whiteheads may result from wind-disseminated ascospores.

WAITE AGRICULTURAL RESEARCH INSTITUTE,
UNIVERSITY OF ADELAIDE,
SOUTH AUSTRALIA.

LITERATURE CITED

1. GRIFFITHS, R. L. Fallowing and cultivation methods for sandy soils. Jour. Dept. Agr. So. Aust. 33: 513–522. 1930.
———. Take-all. Incidence and control on the lighter soils of the mallee. Jour. Dept. Agr. So. Aust. 36: 774–778. 1933.
2. KEITT, G. W., and L. K. JONES. Studies on the epidemiology and control of apple scab. Wis. Agr. Expt. Sta. Res. Bul. 73. 1926.
3. KIRBY, R. S. The take-all disease of cereals and grasses caused by *Ophiobolus cariceti* (Berkeley and Broome) Saccardo. New York (Cornell) Agr. Expt. Sta. Mem. 88. 1925.
4. MCALPINE, D. Take-all and whiteheads in wheat. Dept. Agr. Viet. Bul. 9. 1904.
5. WATERS, R. Take-all disease in wheat. Incidence in New Zealand. New Zeal. Jour. Agr. 20: 137–143. 1920.

WEAK BORDEAUX SPRAY IN THE CONTROL OF FIRE BLIGHT OF APPLE[1]

MONROE McCOWN[2]

(Accepted for publication, Oct. 10, 1932)

Spraying tests for the control of blossom blight of apples and pears, caused by *Bacillus amylovorus* (Burrill) Trevisan, have been conducted in Indiana orchards for the past 6 seasons. The results of earlier tests,[3, 4] showed practical control of the disease by the application of a weak Bordeaux (1–3–50) spray when the blossoms were entering the full-bloom stage. It is the purpose of this paper to present further evidence of the value of Bordeaux in fire-blight control obtained through tests conducted during the past 3 years.

THE EFFECT OF A FULL-BLOOM BORDEAUX SPRAY ON BLOSSOM INFECTION IN INOCULATED GRIMES CLUSTERS

On May 2, 1931, 400 clusters of blossoms on a Grimes apple tree in full bloom in the University orchard at La Fayette were sprayed once with Bordeaux 1–3–50 (1 lb. $CuSO_4$, 3 lbs. hydrated lime, 50 gals. water). The spray was applied by means of a small atomizer. All unopened blossoms were either opened and sprayed or removed, and the wounds thoroughly covered with the Bordeaux spray, to prevent subsequent opening of unprotected blossoms between the time of spraying and the later inoculations. Immediately following the application of the Bordeaux, 100 of the sprayed clusters were inoculated by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. Like numbers were inoculated in the same manner each day during the 3 succeeding days or approximately 24, 48, and 72 hours after spraying. Each day 50 unsprayed blossom clusters were inoculated as checks. The inoculations were made between 10 : 00 a. m. and 3 : 00 p. m. each day. The maximum temperatures recorded by the United States Weather Bureau for May 2, 3, 4, and 5 were 68, 65, 76, and 77 degrees, respectively. No precipitation occurred during

[1] Contribution from the Division of Horticulture, Purdue University Agricultural Extension Service, La Fayette, Indiana.

[2] The writer is indebted to Dr. Max W. Gardner of the Division of Plant Pathology, University of California, Berkeley, California, former Chief in Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana, for valuable advice and suggestions during the progress of the work.

[3] McCown, Monroe. Spraying for the control of fire blight in the apple. Trans. Ind. Hort. Soc. **67**: 129–133. 1928.

[4] McCown, Monroe. Bordeaux spray in the control of fire blight of apple. Phytopath. **19**: 285–293. 1929.

this period, but some rain fell each day from May 6 to May 13 inclusive, totaling 1.15 inches.

The test was repeated in 1932, the technique being the same as that employed in 1932, except that the spraying was done with a small hand sprayer of one quart capacity. All of the blossom clusters on several large branches in two Grimes trees in the University orchard were sprayed May 5, when the clusters were just opening into full bloom. All clusters on one branch were inoculated immediately after spraying by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. The clusters on other branches were similarly inoculated 24 and 48 hours after they were sprayed. Each day the clusters on unsprayed branches were inoculated as checks. The inoculations were made between 10:00 a. m. and 3 p. m. and the maximum temperatures reported for May 5, 6 and 7 were 82, 82, and 77 degrees, respectively. A precipitation of 0.18 inch was recorded on May 4. There was no rainfall May 5 or 6, but 0.82, 0.51, and 0.33 inches of rain fell May 7, 8, and 9 respectively, followed by very light precipitation on May 10, 11, 12, 14, 15, 16, and 17.

During both seasons, records were taken at frequent intervals in order to differentiate between two types of infection: primary, in which the bacteria had entered presumably through the nectary; and secondary, in which the bacteria had entered the base of the pedicel from the spur.

The results, recorded in table 1, show that the Bordeaux was very effective in the prevention of fire-blight infection in blossoms inoculated, both immediately after spraying and 24 hours later. In 1931, while all the check clusters were infected, only 48 per cent of the sprayed clusters inoculated immediately after spraying and 50 per cent of those inoculated 24 hours later blighted. Although 57 per cent of the untreated clusters, inoculated May 5, 1932, were infected only 10 per cent of the sprayed clusters, inoculated on the same day immediately after spraying, blighted. Since less than 5 per cent of either sprayed or check clusters inoculated at the 48- and 72-hour periods in 1931 and 24- and 48-hour periods in 1932 blighted, no records of infection in these clusters were kept. The control obtained in 1931 and 1932, reported in table 1, corroborates the results of the inoculation tests in 1927.

The poorer control obtained in 1927 in clusters inoculated 24 and 48 hours after spraying was, at that time, attributed to the fact that there was an average of one unopened blossom in each cluster at the time the Bordeaux was applied. The results obtained in 1931, *i.e.*, equal control in clusters inoculated immediately after spraying and 24 hours later, following the elimination of this factor, tend to prove this suspicion correct.

A comparison of the average number of primary infections per cluster shows an even greater germicidal action by the Bordeaux than is reflected

TABLE 1.—*Inoculation tests. Effect of Bordeaux mixture 1–3–50 applied as a full-bloom spray upon fire-blight infection in inoculated Grimes apple blossoms*

Year	Time of inoculation	Spray treatment	Number of clusters inoculated	Average no. of primary infections per cluster	Percentage of clusters infected
1931	May 2, immediately after spraying	Bordeaux 1–3–50	93	0.73	48
		none (check)	50	3.52	100
	May 3, 24 hours after spraying	Bordeaux 1–3–50	79	0.78	50
		none (check)	50	2.88	100
1932	May 5, immediately after spraying	Bordeaux 1–3–50	193	0.15	10
		none (check)	159	1.15	57

in the comparison of blighted clusters. In 1927 there was an average of 0.08, 1.08, and 1.25 primary infections per cluster respectively in the sprayed clusters inoculated immediately after spraying, 24 and 48 hours later. The check clusters for these same days yielded 1.46, 2.24, and 1.88 primary infections per cluster. Records for 1931 show an average of 0.73 and 0.78 primary infections per cluster in the sprayed clusters and 3.52 and 2.88 primary infections per cluster in the check clusters inoculated May 2 and 3. In 1932, there was an average of only 0.15 primary infections per cluster in the sprayed clusters and 1.15 in the nontreated clusters.

THE EFFECT OF AN EARLY FULL-BLOOM BORDEAUX SPRAY UPON NATURAL BLOSSOM INFECTION IN JONATHAN APPLE CLUSTERS

A block of Jonathan apple trees in the orchard of the Simpson Orchard Company,[5] near Vincennes, Indiana, was sprayed with Bordeaux 1–3–50 from one side when opening into full-bloom in 1930. A little more than one-half of each tree was thoroughly covered with the spray, and since the application was made with high pressure to well pruned trees of medium size, some of the spray material was forced through the trees and may have provided partial protection to some clusters considered as unsprayed. A row of ten representative trees was selected for records. Unsprayed and treated branches, totaling approximately the same area, on each tree were then selected and the number of infected clusters was counted on each.

[5] The writer wishes to acknowledge the cooperation of the Simpson Orchard Company for the application of the spray; and of Leslie Pierce of the U. S. Bureau of Plant Industry, Vincennes, who assisted in obtaining the data on this test.

The total area represented by the branches selected for records of each treatment approximated one-fifth of the total area of each tree.

The results show that the weak Bordeaux spray was very effective in the control of the disease on these 10 trees. While there was an average of 119 infected clusters on the untreated branches in each tree, the sprayed branches yielded an average of only 39 blighted clusters, resulting in a reduction of 67 per cent in the number of infected clusters as a result of the spray.

These results are in line with the control obtained in 1928 in Jonathan apples and Seckel pears following full-bloom applications of 1–3–50 Bordeaux. The number of infected clusters was reduced 79 and 52 per cent in Jonathan apple and 98 per cent in Seckel pear by the Bordeaux treatment in 1928.

The results obtained in these field tests and in laboratory studies previously reported,[6] in which Bordeaux apparently killed the bacteria, indicate that the Bordeaux spray provides protection to the blossoms through its germicidal action upon the bacteria after they are deposited in the blossom or upon other tissues protected by the spray.

No russetting of the fruit or reduction in the set of fruit has been observed in any of the above tests. In addition, the writer has observed the fruit in various commercial orchards in Indiana which had received a full-bloom application of Bordeaux 1–3–50 and in no case has noticeable injury resulted from the weak Bordeaux spray. No arsenate of lead has been added to the full-bloom sprays used in these tests.

The fact that an early full-bloom spray of Bordeaux 1–3–50 has given practical control of blossom infection by fire blight without apparent injury to either fruit or foliage and may, in some seasons, also prove valuable in the control of apple scab,[7, 8] warrants the use of this spray on blight-susceptible varieties in Indiana orchards.

SUMMARY

Bordeaux 1–3–50 applied early in the full-bloom period reduced fire-blight infection in Grimes blossom clusters inoculated immediately after spraying and 24 hours after treatment. In 1931, while all the inoculated check clusters became infected, only 48 per cent of the sprayed clusters inoculated immediately after spraying and 50 per cent of those inoculated

[6] *Loc. cit.* See footnote 4.

[7] Burkholder, C. L. Dusting *vs.* spraying of apples, 1927–1931. Agr. Exp. Sta. Bul. 356. 1931.

[8] Dutton, W. C. Spraying materials and the control of apple scab. Michigan Agr. Exp. Sta. Spec. Bul. 203. 1930.

24 hours after treatment blighted. In 1932, while 57 per cent of the untreated check clusters blighted, only 10 per cent of the sprayed clusters developed infection. Natural infection in Jonathan clusters in 1930 was reduced 67 per cent by one spray of Bordeaux 1–3–50 applied as the trees were entering the full-bloom period. No apparent injury to fruit or foliage has occurred in these tests following the use of a weak Bordeaux. No reduction in set of fruit has been observed.

Purdue University Agricultural Extension,
Lafayette, Indiana.

OBSERVATIONS AND NOTES ON CITRUS DISEASES IN MINAS GERAES, BRAZIL

ALBERT S. MÜLLER

(Accepted for publication, Oct. 4, 1932)

The citrus industry of Minas Geraes, Brazil, is still in its infancy commercially. In most cases the older farm house plantings are closely spaced and usually show very poor growth conditions, probably because of lack of care. Large commercial orchards are as yet nonexistent in this Province. A considerable amount of experimental plantings of various citrus varieties have been started at the Agricultural College (Escola Superior de Agricultura) opened in 1927. These experimental trees are, therefore, not very old.

TRUNK AND ROOT DISEASES

In many closely spaced farmyard plantings of sweet orange, a gum disease resembling *mal di gomma* is prevalent. This is probably the most important trunk and root disease. This same malady became so serious in a row of 5-year-old seedling grapefruit trees that they had to be removed.

At the Agricultural College, citrus seedlings have been seriously affected with a similar gum disease at the base. Two blocks of Rangpur lime, one of 600 one-year-old seedlings, and the other of 1,000, each showed infections of 31 per cent and 19 per cent, respectively. In 1931 and 1932 5 different stocks, 300 seedlings each, were affected as follows:

Stock	Percentage affected with gum disease	
	1931	1932
Rough lemon	29	23
Rangpur lime	26	19
Sweet orange	26	19
Shaddock	17	11
Sour orange	19	6

In culture tests from 100 seedlings a Phytophthora fungus, probably *Phytophthora parasitica,* was obtained in a number of cases; and also Fusarium and a yellow bacterium. The last 2 organisms were probably secondary, but the pathogenicity of each is being tested.

A root rot has been observed in several localities on tangerine trees with lesions on the upper exposed or nearly exposed roots, with extension of the infection into the base of the trunk. A species of Fusarium has been found spreading as a thin, whitish superficial web over the infected area. In the

cracks of infected bark, abundant perithecia of a Nectria species also were found. Infected bark of roots shreds off easily. The symptoms at the base of the trunk are not unlike common foot-root symptoms, but without gum. The pathogenicity of the 2 organisms is unknown. The disease has been seen on nothing but native tangerines. It may be a form of dry root rot.

Damping-off of seed-bed plants also was troublesome during 2 successive years in Vicosa. Rangpur lime and Rough lemon were the varieties attacked, and the microörganisms associated were found to be species of Fusarium and Phytophthora. The loss during the first year was calculated at 15 per cent and in the second year 5 per cent. No damping-off appeared during the third year. The disease is apparently common in a great many crops here and cannot be disregarded in connection with seed bed work under the conditions that prevail.

DISEASES OF LEAVES, TWIGS, AND FRUIT

Scab.—This can be considered a major disease here. Moderate to heavy infection has been found on the following varieties: Genoa lemon, Rough lemon, Sweet lemon, King tangerine, West Indian lime, Rangpur lime, and the Bitter Sweet. In the case of the last two, 100 per cent infection of the plants has been observed in nursery rows. In the case of the Rangpur, no mature tree has yet been seen that is not severely scabbed on foliage, twig, and fruit. Grapefruit and Sweet orange have not been found affected with scab in Vicosa, but the latter, in the neighboring State of São Paulo, is subject to the disease.

Melanose.—This disease has been seen mostly in old, closely planted patches of seedling orange trees, on the fruit and on the foliage. In the college planting of the "Marfim" lemon it has been especially noticeable, occurring on fruit, foliage, and twigs. On dead twigs in the interior of the trees, pycnidia of the Phomopsis stage have been encountered. Though Phomopsis is considered one of the chief causes of decay of exported Brazilian fruit, no observations have yet been made as to the importance of this phase of the disease here in the interior.

Anthracnose.—This disease, like scab, seems to be present in Minas throughout the year, being found on young twigs and new foliage, in seed beds and in the nursery row, buds, and flowers, young and mature fruits, on mature foliage, but never as a disease resulting in severe losses. Seed-bed infection has reached 5 per cent, while on budded nursery stock, the maximum loss is 10 per cent. The varieties on which a Colletotrichum infection has been found are: grapefruit, shaddock, Rangpur lime, Sweet orange, and particularly Sweet lemon and Genoa lemon. Dying of large areas on leaves, from the tip or spreading from the margin, has been found, especially on Sweet lemons with which is constantly associated the anthrac-

nose organism, *Colletotrichum gloeosporioides*. The disease has never been found causing infection of more than a small percentage of the leaves on the trees. Without doubt there exists here more than one strain of Colletotrichum, since, in cultures, there have appeared distinct forms of *C. gloeosporioides*. No severe cases of dying back have been seen. The disease can be considered as of minor importance, except under special conditions favoring its development. Anthracnose rot of fruits of the lemon and orange and tangerine has been encountered every year, but it is of minor importance.

Felt.—Wherever trees are planted excessively close together it is common to find a brownish white, felt-like fungus living as thick covering over a scale insect on leaves, twigs, or fruits. No direct serious damage seems to be sustained. The felt seems to be very troublesome to remove from the peduncle end of the fruit during packing operations. Dr. Eugenio Rangel has determined this to be *Septobasidium albidum* Pat. and uses the same name for another felty fungus that is of an entirely different color and consistency, which may live unassociated with scale. The latter must be a distinct species. It forms thin, brownish red plates, the center often densely webby. It commonly ties 2 or more adjacent leaves together and is found on twigs and fruits as well as foliage. Like the former it is troublesome to remove from fruit. It is of minor importance.

LEAF DISEASES

Leaf-spotting has been found on old, neglected orange trees with which a fungus of the genus Phyllosticta has been associated. No apparent serious damage to the trees has been observed. Dr. A. Sacca has reported *P. hesperidearum* as causing leaf-spotting in São Paulo and the fungus found here resembles this.

Spotting of leaves of seedling grapefruit trees at Vicosa accompanied by dropping of the affected leaves is often found. Young, as well as mature, foliage is attacked, the leaves yellowing as the spots enlarge or coalesce. The organism associated has been identified by A. Puttemans as *Ascochyta citri* Penz. The disease is of minor importance.

Algal leaf-spot is sometimes encountered on mature foliage. The organism resembles the common *Cephaleurus mycoidea*. The damage is inconsequential. Defoliation has never been seen.

Sooty mold is frequently found here accompanying scale insects. Curling of heavily coated leaves has been observed in nursery rows, this stage really occasioning some damage.

Black Melanose is common in nursery rows under poor growth conditions, but there seems to be no evidence of damage other than the yellowing of the leaves attacked, and this may be due to something else.

FRUIT ROTS

Blue and Green Mold.—Both of the common species of Penicillium, *P. digitatum* and *P. italicum,* are to be found throughout the citrus fruit season here, present on oranges, limes, lemons, grapefruits, citrons, and tangerines. Judging from the nearly complete local lack of understanding of the necessity of care and method during the processes of picking and handling the fruit, the losses due to these fungi could be very high only where Minas fruits entered in commercial channels.

Sour Rot.—Perhaps equally as common as the *Penicillium* spp. has been a species of Oospora, which seems to be identical with *O. citri-aurantii* during the 4 crops so far observed. Bruised, especially fallen, fruit rapidly becomes a watery disagreeable-smelling decayed mass on exposure to the fungus. The organism is frequently encountered producing decay of numerous fruits still on the tree, but in all cases the fruits show positive evidence of previous injury, generally in the form of insect puncture. Artificial inoculations have shown that the fungus produces rapid decay under our conditions.

Aspergillus Rot.—Rot produced by *A. niger* has been observed on bruised and fallen fruits during 2 crops, but not so often as decay occasioned by the already mentioned organisms. It would probably be only a minor cause of fruit rot in transit.

Alternaria Rot.—Decay of satsumas and sweet lemons by an Alternaria species has been observed in one crop season involving only a small amount of fruit.

Fusarium Rot.—Decay of mature limes, principally at the button end, by a Fusarium species also has been observed during the last crop, the percentage of infection being small. In most cases the decaying fruits lay on the ground under the trees, although some fruits with small circular brownish lesions at the stem end were still attached, falling readily when touched.

ENTOMOGENOUS FUNGI

There seem to be 5 equally common fungi parasitic on scale insects of citrus in this region. They may be found throughout the entire year and are important factors in the control of these insects. Vera K. Charles has courteously given the following identifications for them: *Cephalosporium lecanii* Zimm., *Tuberculatia coccicola* Stev., *Myriangium duriaei* Mont., *Podonectria* sp., and *Microcera* sp.

ESCOLA SUPERIOR DE AGRICULTURA,
VICOSA, MINAS GERAES, BRAZIL.

TRANSMISSION OF THE CRINKLE DISEASE OF STRAWBERRY[1]

E. K. VAUGHAN

(Accepted for publication, Oct. 15, 1932)

The crinkle disease of strawberry was recently described by S. M. Zeller and the writer.[2] It was then stated that the disease has the general appearance and behavior of a virosis, being apparently systemic, since runner plants from a diseased parent show disease symptoms and those from a healthy parent appear normal. During the winter of 1931–32 experiments were carried on in the greenhouse to determine the infectious nature of the disease and possible means of transmission.

The strawberry leaf aphid, *Myzus fragaefolii* Cockerell, has previously been shown to transmit yellows[3] and witches' broom,[4] and it was thought the same aphid might also serve in the transmission of the crinkle disease. These aphids accordingly were colonized on 3 strawberry plants of the Marshall variety. All 3 plants (designated as A, B, and C) were greenhouse-grown and showed distinct symptoms of the disease. Twenty aphids were then transferred to each of 5 healthy plants of the same variety, records being kept of the plant from which they were taken. They were allowed to feed here for one week and then removed. The results of these transfers are shown in table 1.

TABLE 1.—*Results of transmission by the strawberry leaf aphid, Myzus fragaefolii*

Plant from which aphids were transferred	A	B	C	Total
Number of plants to which aphids were transferred	19	23	8	50
Number of plants which developed symptoms of crinkle	15	20	7	42
Percentage of plants which developed symptoms of crinkle	78.94[a]	86.95[a]	87.50	84.00
Number of plants on which symptoms of crinkle later disappeared	11[a]	11[a]	1	23
Percentage of plants on which symptoms of crinkle later disappeared	73.33	55.00	14.28	54.76

[a] One plant died after showing symptoms of crinkle.

[1] Published as Technical Paper No. 191 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

A resumé of a thesis presented in partial fulfillment of the requirements for the master's degree from the Oregon State Agricultural College.

[2] Zeller, S. M., and E. K. Vaughan. Crinkle disease of strawberry. Phytopath. 22: 709–713. 1932.

[3] Plakidas, A. G. Strawberry xanthosis (yellows), a new insect-borne disease. Jour. Agr. Res. 35: 1057–1090. 1927.

[4] Zeller, S. M. Preliminary studies on witches' broom of strawberry. Phytopath. 17: 329–335. 1927.

It is of interest that in 23 of the plants more or less marked symptoms appeared and later disappeared so as to make diagnosis practically impossible, while in 17 plants typical symptoms have persisted. (Figure 1.) No investigation has yet been carried on to explain this disappearance of symptoms, nor have these plants, without apparent symptoms, been tested as carriers of the virus.

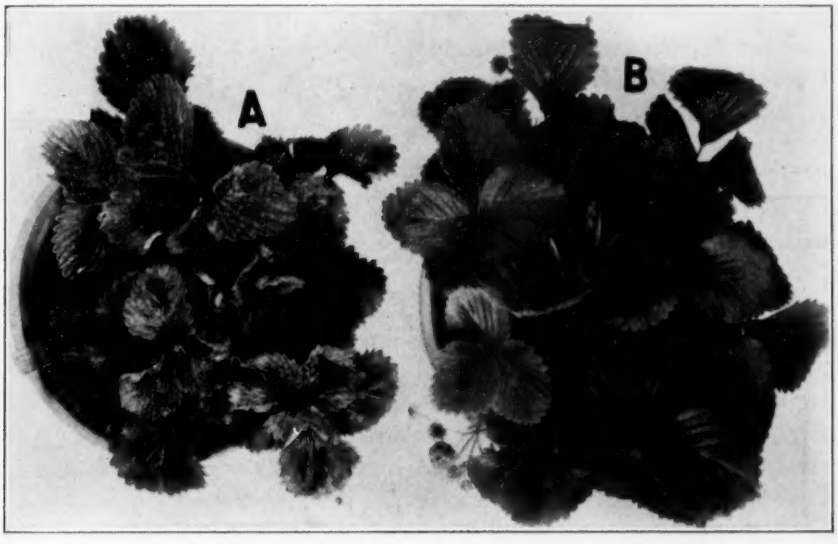

FIG. 1. Marshall strawberry plants of the same age. A. Plant to which crinkle has been transmitted through *Myzus fragaefolii*. B. Healthy plant.

Nonviruliferous aphids also were transferred to healthy plants. These virus-free aphids were obtained by placing adult, agamic female aphids in Petri dishes without food material. Here they were observed at intervals of a few hours until a few young were obtained, which, having had no opportunity to feed were supposedly free of virus contamination. After being allowed to colonize and multiply on a healthy Marshall plant for several weeks, 20 of these aphids were transferred to each of 10 other healthy Marshall plants and allowed to feed for one week. None of these plants showed any symptoms of crinkle after 28 weeks.

Plants of the Ettersburg No. 121 variety, which showed much less pronounced symptoms than the variety Marshall, were thought to have the crinkle disease. Nonviruliferous aphids were colonized for 14 days on a plant of Ettersburg No. 121 having these less marked symptoms. Twenty aphids from the plant were then transferred to each of 10 healthy Marshall plants, where they were allowed to feed for a week. Five of the 10 Mar-

shall plants developed crinkle; in each case only the "pin-point" chlorotic areas described in a previous paper[5] have thus far appeared.

The above experiments have a 4-fold significance: they show, first, that *Myzus fragaefolii* does not produce crinkle directly, but that the viruliferous aphids may act as vectors for the transmission of the infective principle; second, that the infective principle of the crinkle disease of strawberry does not pass from the adult aphids to the young, the first instars; third, that crinkle may be transmitted from one variety to another; *e.g.*, from Ettersburg No. 121 to Marshall; and fourth, that after the first shock from the virus some plants may apparently recover.

Since it is not always possible to detect the symptoms of crinkle in young plants, one could not state definitely that all of the plants used were entirely free of crinkle before having the aphids transferred to them. However, all of them were carefully selected, and as far as could be detected were entirely free of the disease. Of 104 selected Marshall plants obtained in December, 1931, not one has developed crinkle during 34 weeks, unless it was inoculated, while of 24 from this same selection used in the above experiments with viruliferous aphids 14 developed the disease.

No attempts were made to transmit the crinkle disease by means of other insects. *Myzus persicae* Sulzer and an aphid from tobacco were not successfully colonized on strawberry, and the strawberry-root louse, *Aphis forbesi* Weed, was not induced to reproduce during the winter. Mechanical transmission by means of (1) graftage, (2) pricking through diseased leaves into healthy ones, (3) leaf mutilation, and (4) use of extracts from diseased leaves proved unsuccessful.

These experiments were carried on under the direction of Dr. S. M. Zeller, Plant Pathologist, Oregon Experiment Station, whose advice and assistance are sincerely appreciated.

SUMMARY

In this paper are given results of experiments that indicate (1) Crinkle disease of strawberry is caused by a virus, which may be transmitted by the strawberry leaf aphid, *Myzus fragaefolii*. (2) The infective principle does not pass from the adult aphids to the first instars. (3) The disease can be transmitted by aphids from the Ettersburg No. 121 to the Marshall variety. (4) Some plants may recover after the first shock of inoculation. No infection was obtained by such mechanical inoculations as graftage, by leaf mutilation, or by the use of extracts from diseased leaves.

[5] Zeller and Vaughan, *loc. cit.*

OCCURRENCE OF WATERMELON MOSAIC

M. N. WALKER

(Accepted for publication, Sept. 28, 1932)

The natural occurrence of watermelon mosaic was observed early in April, 1932, in Polk County, Florida, near Fort Meade, by the County Agent, F. L. Holland. At that time only 2 or 3 diseased plants were observed in each of 2 fields approximately ¼ mile apart. On April 27, when the writer first inspected these fields, from 20 to 26 affected plants were found in each of several areas along the edges of the fields. Because of the variation in the stages of development of the disease as between plants, it appeared that only 1 or 2 early infected plants in each case had served as the source for secondary infection. The plants outside of these infected areas were in good condition, the vines being nearly full-grown and bearing melons weighing about 15 pounds each.

The diseased vines were very conspicuous because the tips of the runners and a proliferation of shoots from around the crowns protruded stiffly above the general level of the vines, giving what was described by the grower as a "petunia-like" appearance. These tips and shoots showed a shortening of the internodes, resulting in crowding of the young leaves, which appeared somewhat stunted and more rolled than normal. Some mottling was evident on these leaves, the mottling being of a diffuse type, consisting of irregular yellow areas only slightly lighter in color than the normal light green of the young leaves. On some plants these young leaves showed extreme malformation, being reduced in a few cases to almost nothing more than the mid-rib. Older leaves showed a more conspicuous mottling in which dark green raised areas appeared, but the mottling was less characteristic than the severe malformation that occurred on many of the leaves (Fig. 1).

In addition to the crowding of the young leaves on the tips there was also a crowding of flowers and flower buds. Some flowers on the more severely infected plants showed abnormalities of the petals and floral parts, such as unequal lengths and a greenish cast of the petals. The stamens and stigmas were distorted and unequally developed in a few cases. Necrosis and shedding of blossoms occurred before the flowers matured. No melons were set on such plants and the growth and spread of the vines were no more than half of normal vines or vines recently infected. Melons were found on some plants of the latter type, but in most cases they were disfigured by a pronounced mottling of slightly raised, dark green areas. The most pronounced mottling occurred on melons of considerable size

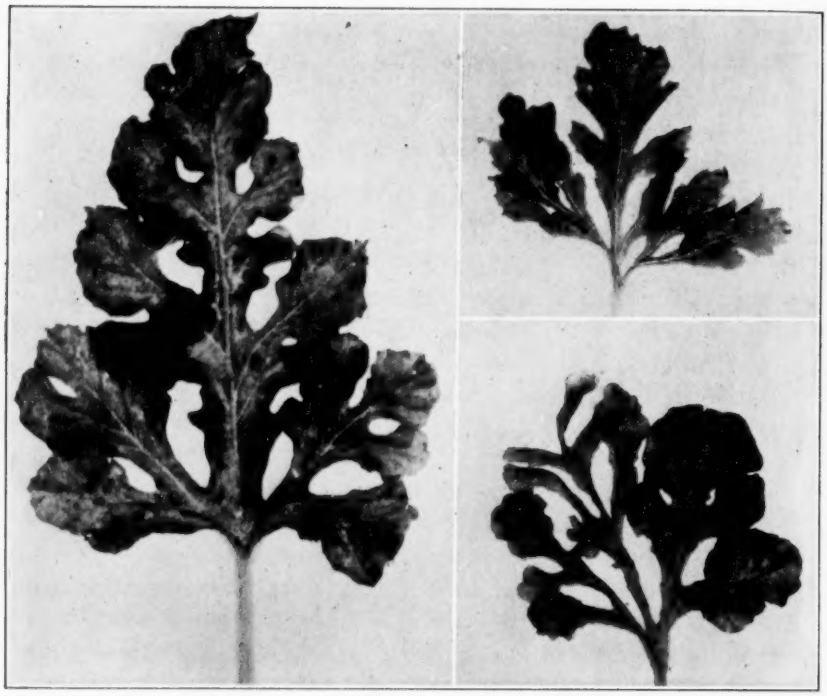

FIG. 1. Leaves from mosaic watermelon plants showing different degrees of mottling and malformation.

(Fig. 2), although small melons were mottled and often malformed. Some of the malformed melons showed pale depressed areas, resembling somewhat the pale areas on the leaves which appear drawn and hard from the concentration of veins. Small blotches of a somewhat paler green than those on other parts of the melon appeared in these areas.

When the fields were inspected a week later, the petunia-like character was less conspicuous, possibly because of the extremely dry weather obtaining at the time. There was some evidence of further spread of the disease, however, and infected plants were found scattered over a 30-acre field, which showed the worst early infection. This field showed an average of 1 per cent infected plants, most of which were concentrated in 4 areas in different parts of the field. Comparatively few melons were found in these areas and most of them were disfigured. Another field of 70 acres showed one badly diseased area with an occasional infected plant near-by. These 2 fields had survived the cold of early March, and were further advanced than a third near-by field of about 15 acres that was planted after the cold spell. A single diseased plant was found in the latter. Another field of about 30

FIG. 2. Half-grown melon showing severe disfiguration as a result of mosaic infection.

acres, 3 miles from the other 3 fields, showed an area that contained about 20 definitely mosaic plants and a few others that were probably infected with mosaic, although they showed nothing more than a general yellowing. All 4 fields were planted to the Tom Watson variety of melons.

The agency of transmission has not been determined but there was an unusually heavy infestation of aphids, *Aphis gossypii*, in the state during the spring, and opportunities for the spread of mosaic by this insect were abundant. A close survey was made of the immediate vicinity of the field showing the greatest number of mosaic plants and no diseased wild plants were found, but 4 species of plants that might serve as wild hosts were found abundantly in and around the diseased field. These plants were 2 species of Physalis: *P. ciliosa*, a perennial, and *P. angulata*, an annual; one species of Phytolacca, *P. rigida;* and an unidentified species of Asclepias. No cultivated crops other than sweet potatoes had been grown in the vicinity of these fields during the preceding year.

This discovery of watermelon mosaic in the field was surprising since no reports have been found in literature on the natural occurrence of watermelon mosaic. One of the oldest growers in Polk County stated that he had seen nothing of similar nature during his 25 years' experience as a watermelon grower in that vicinity. Furthermore, only 3 reports have been found on the successful transmission of mosaic to watermelon plants. Jagger reported[1] the infection of 2 watermelon plants of the variety Cole's

[1] Jagger, I. C. Experiments with cucumber mosaic. Phytopath. 6: 148–151. 1916.

Early with a virus from cucumber plants. Porter described[2] a mosaic disease of cucumbers caused by cucumber Virus 2 which he successfully transmitted to several varieties of watermelons. Porter and Melhus reported[3] the F_1 generation of certain watermelon-citron crosses to be highly susceptible to mosaic.

It is possible that this occurrence of watermelon mosaic[4] was the result of an unusual combination of certain factors which may not be properly combined again for several years. However, since the disease showed such potentialities for injury, a close watch should be kept for its recurrence.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLORIDA.

[2] Porter, R. H. The reaction of cucumbers to types of mosaic. Iowa State Coll. Jour. Sci. 6: 95–129. 1931.

[3] Porter, D. R., and I. E. Melhus. The pathogenicity of *Fusarium niveum* (E F S.) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad). Iowa Agr. Exp. Sta. Res. Bul. 149. 1932.

[4] No report of the occurrence of this disease has been received during the 1933 season.

PHYTOPATHOLOGICAL NOTE

Humus Extract Agar favorable for Oospore Production in Pythium.—The frequent absence of the sexual stage on ordinary culture media is a widely recognized handicap in identification of certain species of Pythium. Working with a collection of *P. arrhenomanes* Drechsler, from root rot of sugarcane, the writers tested a large number of ordinary and special laboratory media with, in some cases, variation in temperature and pH, as well as the effect of mixing with bacteria and molds. Never more than 24 per cent of the strains produced oospores. The latter, when present, were usually more abundant and normal in appearance on the grated carrot agar suggested by Miss Johann.[1] This was especially noticeable in the culture of *P. arrhenomanes* studied by her and used also in these tests.

What seemed to be oospores of one of the recalcitrant strains had been observed in infected cane roots in an advanced stage of decay. Therefore, soil and humus extracts (so-called humic acid) were added to the carrot medium. Transfers to this have resulted in the development of normal oospores by 95 per cent of the isolates, making possible their definite identification. Although a given culture has not fruited every time, it always has upon one or more replatings.

The humic acid used largely by the writers was extracted from a local woodland soil according to the method described by Burk, Lineweaver and Horner.[2] Satisfactory sporulation, however, has been obtained in the presence of humus extracts from two other widely different sources, *viz.*, from a fertile garden soil and from decomposed filter-press cake, a 50 per cent organic residue from the clarification of sugarcane juice. The humic acid at the rate of 50 p.p.m. (organic content) is added to cornmeal agar, which is then tubed and the grated carrot added to each tube before autoclaving, as suggested by Johann. Carrot or other vegetable tissue is usually essential. Synthetic humic acids prepared from dextrose with and without added iron, and ferric citrate alone, did not stimulate oospore development. —R. D. Rands and Ernest Dopp, Division of Sugar Plant Investigations, Bureau of Plant Industry, Washington, D. C.[3]

[1] Johann, Helen. Grated carrot agar favorable for studies of Pythium. (Note) Phytopath. **18**: 710. Aug. 1928.

[2] Burk, D., H. Lineweaver, and C. K. Horner. Iron in relation to the stimulation of growth by humic acid. Soil Sci. **33**: 413–453. June 1932.

[3] The writers are indebted to Messrs. Burk, Lineweaver, and Horner for helpful suggestions, a few of the humic acids tested, and for determinations of organic matter in others.

A Ringspot-like Virus Disease of Red Clover. In September, 1931, a virus disease of red clover, *Trifolium pratense*, differing from the mosaic disease usually described, was seen in a small planting in the rear of the Kentucky Agricultural Experiment Station greenhouse. A number of plants in each of 4 clover strains, Kentucky, Oregon, Wisconsin, and French, in this area, were affected by this disease.

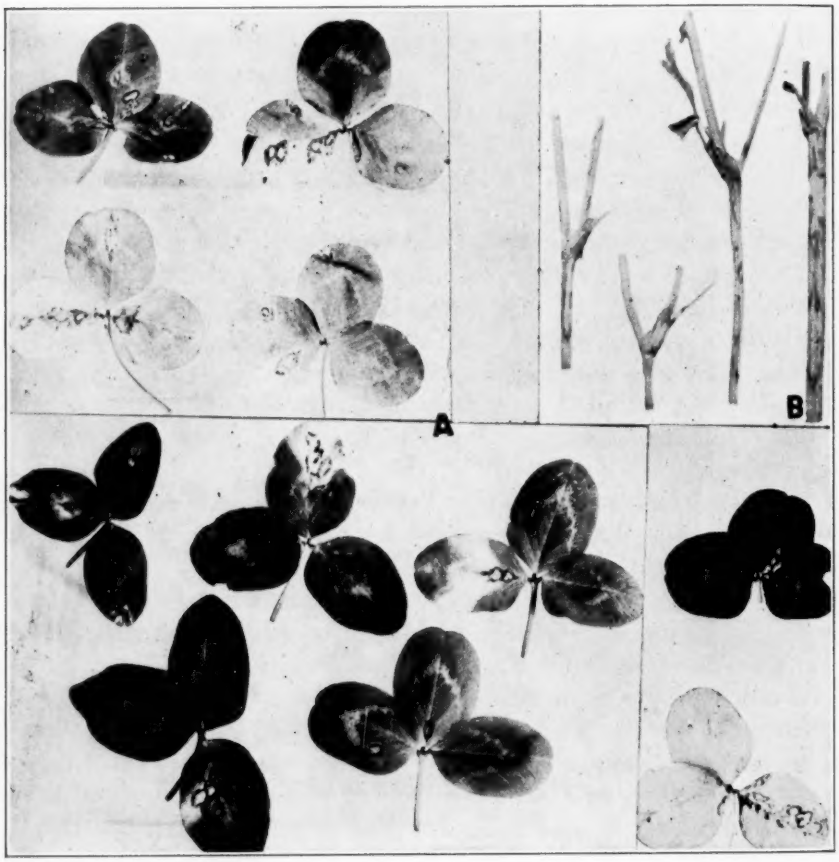

FIG. 1. Ringspot-like virus disease of red clover. A. On the leaflets yellow, circular areas of various sizes with necrotic borders develop. Some leaflets thus affected lose nearly all of the green color and assume a lemon-yellow appearance. B. On stems appear yellow ring-like patterns with brownish borders.

Small, irregular to circular, yellowish spots from a few millimeters to 2 cm. in diameter, with well-defined necrotic borders were observed. (Fig. 1, A.) The chlorosis or yellowing in some spots spread out irregularly

beyond the necrotic border and sometimes one or more leaflets were entirely yellowed. In these the necrotic, ring-like areas were always clearly visible. Long, narrow, irregularly oval, yellow areas with necrotic borders were present on some of the stems of affected plants. (Fig. 1, B.) Usually only a few leaves of a plant were affected and the vigor of the plant did not seem lowered.

The spots resembled those of yellow ringspot of tobacco and a row of tobacco affected with yellow ringspot grew a few feet from the clover. Attempts to transmit the clover disease to 27 Turkish tobacco plants by rubbing juice of affected clover on the leaves by means of sterile swabs were unsuccessful. Aphids were transferred to Turkish tobacco plants from ringspot clover plants. Although a number of transfers were made to the same plants, the insects always died within 4 or 5 days. Even though no symptoms developed in these tobacco plants, inoculations were made from them to healthy tobacco plants, but with negative results.

The garden bean, *Phaseolus vulgaris,* is susceptible to tobacco ringspot. Five bean plants inoculated with juice from diseased clover developed no recognizable symptoms. The proximity of the affected clover plants to tobacco plants affected with yellow ringspot and the close correspondence in symptoms suggest an identical cause, yet inoculation tests leave this question open.

Diseased clover plants were marked and seed harvested in October. Two hundred and fifty-five seeds from 5 plants were sown in flats from which 165 seedlings developed. No symptoms of disease were observed in any seedlings. This disease was seen for the first time in 1931 and only in a small plot. The writer was unable to find the disease in extensive strain tests of red clover on the experiment station farm in 1931–1932.—E. M. JOHNSON, Kentucky Agricultural Experiment Station, Lexington, Ky.